RC
267
U5626
pt.3B
1977

U.S. NATIONAL CANCER INSTITUTE

ANNUAL REPORT OF PROGRAM ACTIVITIES

July 1, 1976 through September 30, 1977

Part III B

DIVISION OF CANCER CAUSE AND PREVENTION

Viral

U.S. National Cancer Institute
" Report of program activities

PART III-B

SUMMARY REPORT

OFFICE OF THE ASSOCIATE DIRECTOR, VIRAL ONCOLOGY PROGRAM

July 1, 1976 - September 30, 1977

Dr. J. B. Moloney

The Annual Report for the Viral Oncology Program, Division of Cancer Cause and Prevention, NCI is presented as follows:

A. Viral Oncology Program (VOP) - Virus Cancer Program (VCP)

1. Introduction
2. Organization
   a. Viral Oncology Program (VOP)
   b. Virus Cancer Program (VCP)
3. Administrative Highlights
4. Scientific Activities
   a. Narrative
   b. Progress Highlights
5. Projections
6. Meeting Reports
7. Bibliography

B. Summary Reports - Viral Oncology Program

1. Offices of the Associate Director, Viral Oncology Program
   a. Office of Biohazard Safety
   b. Office of Program Resources and Logistics
   c. Frederick Cancer Research Center
   d. Editorial Unit
2. Laboratory Reports
   a. Laboratory of DNA Tumor Viruses
      (1) Summary
      (2) Individual Project Reports
   b. Laboratory of RNA Tumor Viruses
      (1) Summary
      (2) Individual Project Reports

C
67
5626
1.3B
977

c. Laboratory of Tumor Virus Genetics

(1) Summary
(2) Individual Project Reports

d. Laboratory of Viral Carcinogenesis
(1) Summary
(2) Individual Project Reports

C. Summary Reports - Virus Cancer Program

1. Collaborative Research Branch

a. Branch Summary
b. Section Summaries and Contract Reports

(1) Breast Cancer Virus Studies
(2) Clinical Studies
(3) Cocarcinogenesis Studies
(4) DNA Virus Studies
(5) RNA Virus Studies

2. Office of Program Resources and Logistics Contract Reports

3. Program Management Contract and Grant Reports

a. Cancer Research Emphasis Grants (CREG)
b. Contract Reports

A. Viral Oncology Program (VOP) - Virus Cancer Program (VCP)

1. Introduction

The Viral Oncology Program (VOP) is responsible for planning and conducting the Institute's program of coordinated research on the role of viruses in cancer. Scientists within the VOP conduct laboratory investigations and assist in the management of a collaborative program, the Virus Cancer Program (VCP). These Programs have the following goals: (1) to search for viruses or virus genetic information related to the initiation of human cancer; (2) to elucidate the process by which normal cells become malignant using viruses as probes; and (3) to develop preventive and therapeutic measures for the control of human cancer.

Since its initiation 13 years ago, the Virus Cancer Program has brought together investigators from around the world to focus their skills on the expansion of knowledge on the oncogenic properties of viruses and their relationship to human neoplasia. The Program has brought visibility to this problem area, resulting in an increased understanding of the nature of the interactions between viruses and cells. It has opened new vistas for biological investigation and has accelerated new developments far beyond

its limited scope of activity. An awareness of the value of tumor viruses as tools for the study of biological processes now exists which had not been perceived when the Program was begun. We now know that different viruses can cause cancer in different mammalian species ranging from mice to large domestic animals and monkeys, and that such viruses may be transmitted through germ cells as well as from animal to animal. Highly specific probes detect virus-related components in human cells. Techniques under development may improve diagnosis and prognosis for certain human cancers. The practical nature of much of this work was accomplished under this contract Program; first as the Special Virus-Leukemia Program, then the Special Virus-Cancer Program, and now the Virus Cancer Program.

The yearly budgets from the inception of a targeted Program in 1964 to the present and the number of professional positions are given in Table 1.

2. Organization

a. Viral Oncology Program (VOP)

The Viral Oncology Program (VOP) and the Virus Cancer Program (VCP) have been organized as an integrated structure. The Office of the Associate Director, Viral Oncology Program, is responsible for the coordination of both the intramural and the collaborative research programs in cancer virology. The Program now has four Laboratories and one Branch. Each of these subdivisions has several sections, as listed in the Summary of Intramural Organization. The term "laboratory" is now used to describe the major intramural subdivisions. The term "branch" refers exclusively to the extramural or collaborative program. The functional statements for each Office, Laboratory and Branch are given below; functional statements for Sections are found in the Laboratory reports. In general the names of each Laboratory reflect the major research mission.

This year the Co-Carcinogenesis Studies Section was created within the Collaborative Research Branch to manage contracts on the interaction of viruses and environmental agents as cofactors for the transformation of cells to malignancy. Two new Sections were accommodated in the intramural laboratories: the Cellular Transformation Section was transferred from the Carcinogenesis Program, DCCP, to the Laboratory of Tumor Virus Genetics, VOP, DCCP; and the In Vitro Carcinogenesis Section was transferred from DCBD to the Laboratory of RNA Tumor Viruses, VOP, DCCP.

Functional Statements

Viral Oncology Program. (1) Plans, directs, coordinates, and evaluates a program of basic and applied research on viruses as etiological agents of cancer; (2) establishes program priorities, allocates resources, integrates the projects of the various branches, evaluates program effectiveness, and represents program area in management and scientific decision-making meetings within the Institute; (3) administers research in biochemistry, tumors,

genetics, pathology, biohazards, immunology, the environment, and viral and cell biology through intramural laboratories and contracts; (4) advises the Director of the Division and supports the activities of the National Cancer Advisory Board and other scientific advisory committees.

Office of Program Resources and Logistics. Plans, initiates and maintains a coordinated program to anticipate and meet the needs of the Viral Oncology Program (intramural and collaborative) for research resources and logistical support, including selection of appropriate contractors and management of contracts and the distribution of research materials. Develops and maintains a computerized inventory and management information system for the resources acquired and used by the Program.

Office of Biohazards Safety. Performs research in virology, aerobiology, mammalian physiology, and biochemistry to evaluate the risk to the host when exposed to infectious agents. Develops and recommends equipment and procedures for handling of potentially biohazardous materials and disseminates this information to the interested scientific community throughout the world.

Collaborative Research Branch. Participates in the planning, development, and scientific administration of a program of collaborative research conducted within the Virus Cancer Program on viruses as etiologic agents of cancer in man and on the control of tumor viruses and/or their induced diseases.

Laboratory of RNA Tumor Viruses. Plans and conducts research on the role of endogenous RNA viruses in natural and induced cancers of animals and man. Develops and applies methods for the prevention and control of cancers by vaccination with RNA viruses and by treatment with inhibitory host cell factors. Studies mechanisms of tumor suppression by chemotherapeutic and immunotherapeutic agents.

Laboratory of DNA Tumor Viruses. Plans and conducts research on DNA viruses to define their role in the development of cancers in animals and man. Develops and applies biological, biochemical, and immunological procedures to obtain evidence for virus genetic expression in neoplastic cells. Conducts investigations to determine the mechanisms by which cellular gene expression, viral gene expression, and interactions between viruses influence transformation of cells.

Laboratory of Tumor Virus Genetics. Plans and conducts research to determine the molecular basis for the etiology of cancers. Conducts studies using molecular techniques to detect viral genes responsible for oncogenesis and to prevent their action. Develops assays for detection and characterization of gene products involved in cellular transformation to malignancy. Elucidates the mechanisms by which the host regulates the expression of oncogenic viruses.

Laboratory of Viral Carcinogenesis. Plans and conducts research on virus-host relationships in virus-induced cancers with emphasis on the detection and characterization of oncogenic viruses and the mode of viral transmission in animals and man. Studies the interactions of viral and cellular genes. Studies host immune mechanisms related to the control of virus-induced cancers. Conducts investigations on molecular processes in viral carcinogenesis.

Table 1

FUNDING HISTORY OF THE VIRAL ONCOLOGY PROGRAM

(in thousands)

Fiscal Year	Positions	In-House	VO Contracts	SVLP	VCP	BCTF	CREG	TOTALS
1964	30	—	4926	—	—	—	—	
1965	117	1687	5433	8723	—	—	—	15,843
1966	140	1835	3064	13,556	—	115	—	18,570
1967	144	1999	3137	13,505	—	246	—	18,887
1968	157	2239	—	—	17,241	284	—	19,764
1969	176	2891	—	—	17,985	259	—	21,135
1970	180	3356	—	—	17,340	174	—	20,870
1971	197	4517	—	—	31,591	234	—	36,342
1972	226	6310	—	—	41,889	734	—	48,933
1973	219	6983	—	—	42,564	1006	—	50,553
1974	231	7189	—	—	49,553	1150	—	57,892
1975	229	9395	—	—	49,387	1450	—	60,232
1976	222	10,800	—	—	46,773	1450	967	59,990
1977	234	12,547	—	—	44,450	1450	1800	60,247

Summary of Intramural Organization

- OFFICE OF THE ASSOCIATE DIRECTOR, VIRAL ONCOLOGY PROGRAM
  - Office of Program Resources and Logistics
  - Office of Biohazard Safety
    - Biohazards Research Section
  - Collaborative Research Branch
    - Breast Cancer Virus Studies Section
    - Clinical Studies Section
    - Co-carcinogenesis Studies Section
    - DNA Virus Studies Section
    - RNA Virus Studies Section
  - Laboratory of RNA Tumor Viruses
    - Animal Immunotherapy Section
    - In Vitro Carcinogenesis Section
    - Molecular Biology Section
    - Viral Genetics Section
    - Viral Immunology Section
    - Viral Immunotherapy Section
    - Virus Disease Modification Section
  - Laboratory of DNA Tumor Viruses
    - Cell Physiology Section
    - Microbiology Section
    - Primate Virus Section
    - Virus Tumor Biochemistry Section
    - Virus Tumor Biology Section
  - Laboratory of Tumor Virus Genetics
    - Cellular Transformation Section
    - Molecular Virology Section
    - Viral Biochemistry Section
    - Viral Immunogenetics Section
  - Laboratory of Viral Carcinogenesis
    - Cell Biology Section
    - Immunology Section
    - Tumor Virus Detection Section
    - Ultrastructural Studies Section
    - Viral Leukemia and Lymphoma Section
    - Viral Pathology Section
    - Virus Control Section

Intramural Review Committees

Virus Cancer Program Coordinating Committee

Dr. Stuart Aaronson
Dr. James Duff
Dr. Peter Fischinger
Dr. Berge Hampar
Dr. David Howell, Executive Secretary
Dr. Robert Huebner
Dr. Robert Manaker
Dr. J.B. Moloney, Chairman
Dr. Wade Parks
Dr. Jeffrey Schlom
Dr. Edward Scolnick
Dr. Louis Sibal, Vice-Chairman
Dr. George Todaro
Dr. George Vande Woude

Viral Oncology Program Resources & Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. Garrett Keefer, Executive Secretary
Dr. Peter Fischinger
Dr. Clarice Gaylord
Dr. Maurice Guss
Dr. Takis Papas
Dr. Ernest Plata
Dr. John Stephenson
Dr. David Troxler
Dr. George Vande Woude
Dr. David West
Dr. Wilna Woods

Clinical Advisory Group for Viral Oncology

Dr. Michael Blaese (DCBD)
Dr. Peter Fischinger (DCCP)
Dr. Robert Gallo (DCT)
Dr. Sylvan Green (DCCP)
Dr. Curtis Harris (DCCP)
Dr. Ronald Herberman (DCBD)
Dr. Paul Levine (DCCP)-Chairman
Dr. Franco Muggia (DCT)
Dr. Alan Rabson (DCBD)

Contract Specialists (Research Contracts Branch)

*Mr. William Caulfield
Mrs. Dorthy Coleman
**Mr. Charles Fafard
Mr. Sidney Jones
Mr. Jacques Labovitz
Mr. J. Thomas Lewin
Mr. Thomas Porter
Mr. Clyde Williams

---

*Deputy Chief
**Chief

Virus Cancer Program Scientific Review Committee A

Dr. David Bishop, Rutgers University (New Brunswick, NJ)
Dr. Arthur Brown, University of Tennessee (Knoxville, TN)
Dr. Andrew Dean, Arkansas Department of Health (Little Rock, AR)
Dr. Donald Fine, Frederick Cancer Research Center (Frederick, MD)
Dr. Eva Klein, Karolinska Institutet (Sweden)
Dr. Mathilde Krim, Sloan-Kettering Institute for Cancer Research (NYC)
Dr. Arnold Levine, Princeton University (Princeton, NJ)
Dr. Miriam Lieberman, Stanford University Medical School (Stanford, CA)
Dr. Frank Lilly, Albert Einstein College of Medicine (Bronx, NY)
Dr. William C. Moloney, Peter Bent Brigham Hospital (Boston, MA)
Dr. Richard Morrow, Harvard School of Public Health (Boston, MA)
Dr. Garth Nicolson, Salk Institute (San Diego, CA)
Dr. Malcolm C. Pike, USC School of Medicine (Los Angeles, CA)
*Dr. William Rawls, McMaster University Medical School (Canada)
Dr. Philips Robbins, Massachusetts Institute of Technology (Cambridge, MA)
Dr. Kenneth Takemoto, NIAID, NIH
Dr. Lise Thiry, Institute Pasteur du Brabant (Belgium)
Dr. Adel A. Yunis, University of Miami School of Medicine (Miami, FL)

Dr. Maurice L. Guss, NCI, NIH (Acting Executive Secretary)

Virus Cancer Program Scientific Review Committee B

Dr. J. Thomas August, Albert Einstein College of Medicine (Bronx, NY)
**Dr. Paul H. Black, Massachusetts General Hospital (Boston, MA)
Dr. George Blumenschein, University of Texas Medical School (Houston, TX)
Dr. Janet S. Butel, Baylor University College of Medicine (Houston,TX)
Dr. Robert M. Chanock, NIAID, NIH
Dr. Joseph Feldman, Scripps Clinic & Research Foundation (La Jolla, CA)
Dr. Fred C. Fox, University of California (Los Angeles, CA)
Dr. Clarence J. Gibbs, Jr., NINCDS, NIH
Dr. Ronald Glaser, Pennsylvania State University (Hershey, PA)
Dr. Adeline J. Hackett, University of California (Berkeley, CA)
Dr. Francoise Haguenau, College de France (Paris)
Dr. Jacob Holper, Litton Bionetics, Inc. (Kensington, MD)
Dr. Albert Kaplan, Vanderbilt University (Nashville, TN)
Dr. Diana Lopez, University of Miami (Miami, FL)
Dr. Henry T. Lynch, Creighton University School of Medicine (Omaha, NB)
Dr. Gary R. Pearson, Mayo Clinic (Rochester, MN)
Dr. Marvin A. Rich, Michigan Cancer Foundation (Detroit, MI)

Dr. Wilna A. Woods, NCI, NIH (Acting Executive Secretary)

---

*Chairman, Committee A
**Chairman, Committee B

CONSULTANTS

Dr. Roland Aronson, Johns Hopkins University, Baltimore, MD
Dr. Giuseppe Attardi, California Institute of Technology, Pasedena, CA
Dr. Thomas August, Albert Einstein College of Medicine, Bronx, NY
Dr. Richard Axel, Columbia University, New York, NY
Dr. Ninarayna Battula, McArdle Institute, Madison, WS
Dr. Arthur L. Beaudet, Baylor College of Medicine, Houston, TX
Dr. George Bekesi, Mt. Sinai Hospital and School of Medicine, New York, NY
Dr. Paul Black, Massachusetts General Hospital, Boston, MA
Dr. Dani Bolognesi, Duke University Medical Center, Durham, NC
Dr. F.J. Bollum, University of Kentucky Medical Center, Lexington, KY
Dr. Michael Botchan, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
Dr. Soh Ha Chan, WHO Immunology Center, Singapore 3
Dr. Carlo M. Croce, Wistar Institute, Philadelphia, PA
Dr. Minoo N. Dastoor, University California, Los Angeles, CA
Dr. Jack Dean, Litton Bionetics, Inc., Kensington, MD
Dr. Walter Eckhart, Salk Institute for Biological Studies, San Diego, CA
Dr. Yael Eilam, Weizmann Institute of Science, Rehovat, Israel
Dr. Marvin Frazier, ERDA, Richland, WA
Dr. Charlotte Friend, Mt. Sinai School of Medicine, New York, NY
Dr. Raymond V. Gilden, Frederick Cancer Research Center, Frederick, MD
Dr. Anthony Girardi, East Tennessee Cancer Research Center, Knoxville, TN
Dr. Adolph Graessman, Freie Universitat, West Berlin, Germany
Dr. Hidesaburo Hanafusa, Rockefeller University, New York City, NY
Dr. William Hardy, Sloan-Kettering Institute for Cancer Research, New York, NY
Dr. Gloria Heppner, Roger Williams General Hospital, Providence, RI
Dr. Nancy Hopkins, Massachusetts Institute of Technology, Cambridge, MA
Dr. Robert Hunter, University of Chicago, Chicago, IL
Dr. Warren Jelenek, Rockefeller University, New York City, NY
Dr. Fred Jensen, Scripps Clinic and Research Foundation, La Jolla, CA
Dr. Anna Kadish, Albert Einstein College of Medicine, Bronx, NY
Dr. Faras Kafatos, Biological Laboratories, Harvard College, Cambridge, MA
Dr. Edmund Klein, Roswell Park Memorial Institute, Buffalo, NY
Dr. Patrick Lai, Yale University, New Haven, CT
Dr. Boris Lapin, Sukhumi Institute of Experimental Pathology & Therapy, Sukhumi, USSR
Dr. Edwin Lennette, Department of Public Health, Berkeley, CA
Dr. Arleen C. Levin, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL
Dr. Arnold Levine, Princeton University, Princeton, NJ
Dr. Diana Lopez, University of Miami, School of Medicine, Miami, FL
Dr. Tom Maniatis, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
Dr. Charles McGrath, Michigan Cancer Foundation, Detroit, MI
Dr. William McGuire, University of Texas Health Science Center, San Antonio, TX
Dr. Dan Medina, Baylor University College of Medicine, Houston, TX
Dr. Robert C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, WA
Dr. Fred Rapp, Pennsylvania State University, Hershey, PA
Dr. William Riggsby, University of Tennessee, Knoxville, TN

Dr. Rex Risser, University of Wisconsin, Madison, WS
Dr. Frank H. Ruddle, Yale University, New Haven, CT
Dr. Joseph Sambrook, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
Dr. Robert C. Sawyer, Rockefeller University, New York City, NY
Dr. Sol Spiegelman, Institute for Cancer Research, Columbia University, New York, NY
Dr. Richard Steeves, Albert Einstein College of Medicine, Bronx, NY
Dr. Gerald V. Stokes, Meharry Medical College, Nashville, TN
Dr. John Taylor, Institute for Cancer Research, Philadelphia, PA
Dr. Josh C. Tunca, West Virginia University Medical Center, Morgantown, WV
Dr. John Verna, Meloy Laboratories, Inc., Springfield, VA
Dr. Robert Wagner, University of Virginia School of Medicine, Charlottesville,VA
Dr. Robert Weinberg, Massachusetts Institute of Technology, Cambridge, MA
Dr. W.S.M. Wold, Saint Louis University of Medicine, Saint Louis, MO

Virus Cancer Program Advisory Committee

Dr. Charlotte Friend, Mt. Sinai School of Medicine, New York NY
*Dr. Anthony Girardi, East Tennessee Cancer Research Center, Knoxville, TN
Dr. Edmund Klein, Roswell Park Memorial Institute, Buffalo, NY
Dr. Daniel Nathans, Johns Hopkins University School of Medicine, Baltimore, MD
Dr. Lloyd Old, Sloan-Kettering Institute, New York, NY
**Dr. Frank W. Putnam, Indiana University Medical School, Bloomington, IN
*Dr. Jack Strominger, Sidney Farber Cancer Institute, Boston, MA
/Dr. Robert Wagner, University of Virginia Medical School, Charlottesville, VA

(Dr. David Howell, Executive Secretary)

---

*Appointment effective July 1, 1977

**Chairman

/Term expires 1977

b. The Virus Cancer Program (VCP)

Background. At the present time the Virus Cancer Program is administered by the Collaborative Research Branch with five Sections: the Breast Cancer Virus Studies Section; the Clinical Studies Section; the Co-carcinogenesis Studies Section; the DNA Virus Studies Section; and the RNA Virus Studies Section. Virus-cancer research is now managed by the heads of each Section under the general supervision of the Branch Chief, the Associate Chief, and the Office of the Associate Director; Section heads no longer conduct intramural research.

Advice on overall Program direction is now provided by the VCP Advisory Committee, consisting of seven members including the Chairman. The members are chosen entirely from the biomedical community on the basis of their qualifications and expertise in the cancer field, in particular cancer virology. The Committee has met on several occasions to advise on areas for expanded research and development, and the application of research findings to the control of cancer in man. The Committee's efforts are separate from the review and approval of individual contracts.

Review of Contracts. Projects within the total Program are reviewed at many levels:

(1) Each contract is reviewed for relevance, priority and need to the total Program by the Virus Cancer Program Coordinating Committee (VCPCC). This Committee, composed of members of the Office of the Associate Director, the Collaborative Research Branch, and senior intramural investigators, reviews solicited and unsolicited proposals developed within the Program. Consensus is obtained by open vote and priority is established by scoring on secret ballot.

(2) Each contract is reviewed for scientific excellence and technical competence by Scientific Review Committee A or B. These committees, first chartered in FY 1975, consist of up to 25 members each, including the Acting Chairman. The members were appointed by the Director, NCI, to serve overlapping terms of four years on the basis of their outstanding qualifications in the fields of microbiology, molecular biology, immunology, biochemistry, virology, and clinical medicine, as related to cancer. The committees are composed entirely of non-NCI scientists. Although intramural scientists participate as needed in the review process, they are not voting members. The committees operate entirely under the regulations set forth by the OMB, DHEW, NIH, and NCI, with an Executive Secretary who is responsbile for the review and complete documentation of each project.

(3) Each contract is continually monitored for performance by (a) the Associate Director's Science Management Team; (b) the Collaborative Research Branch; (c) CRB Section Heads; (d) Project Officer(s). Project Officers are intramural senior laboratory investigators who serve as advisers to principal investigators on scientific matters.

(4) Each contract above the annual funding level of $1 million and with multifaceted workscope undergoes a third review by an ad hoc committee appointed by the Associate Director, Viral Oncology Program.

(5) Each contract is reviewed by the Associate Director, Viral Oncology Program; the Director, Division of Cancer Cause and Prevention; the Chief, Research Contracts Branch, NCI; and, as required, by the Director, NCI.

As an aid to the review processes, key staff members receive progress reports on all contracts on a biannual basis. Collection and distribution of these reports is the responsibility of the Editorial Unit in the Office of the Associate Director. A single comprehensive report is prepared annually by the Associate Director.

Flow Plan for Solicited Proposals.

(1) Project idea conceived and developed by Program.

(2) Coordinating Committee review. (Originator of plan presents to Committee for determination of relevance, priority, and need.)

(3) Project Plan document and RFP drafted. (Originator and Contract Specialist prepare the Project Plan and the RFP.)

(4) Technical review and contractor selection. (Competitive processes completed as required. Scientific Review Committee reviews technical aspects and ranks competing proposals. Program staff selects contractor(s).

(5) Project Plan document approved and Review Summary Sheet prepared and approved. (Project Officer and Contract Specialist prepare Review Summary Sheet and route Project Plan and Review Summary Sheet for signatures.)

(6) Negotiation and award. (Contract Specialist negotiates contract with help of Project Officer as required. Preliminary discussions and negotiations may have occurred under Contractor Selection and Technical Review.)

Flow Plan for Unsolicited Proposals.

(1) Unsolicited proposal received by Program.

(2) Coordinating Committee review. (Coordinating Committee determines relevance, priority, need and uniqueness.)

(3) Project Plan drafted. (Project Plan document, Justification for Non-Competitive Procurement, prepared by Program staff member and Contract Specialist.)

(4) Technical review. (Technical review of proposal performed by Scientific Review Committee.)

(5) Project Plan and Review Summary Sheet approved. (Contract Specialist routes Project Plan document and Review Summary Sheet for signatures.)

(6) Negotiation and award. (Contract Specialist negotiates contract with help of Project Officer as required.)

Scientific Staff, Office of the Associate Director, Viral Oncology Program

Dr. J.B. Moloney, Associate Director
Dr. L.R. Sibal, Deputy Associate Director
Dr. D.M. Howell, Assistant to the Deputy Associate Director
Dr. H.J. Hearn, Scientific Coordinator for Viral Oncology, Frederick Cancer Research Center (FCRC)

Science Management Team, Office of the Associate Director, VOP

Dr. J.T. Duff
Mr. Charles Fafard
Dr. D.M. Howell
Dr. R.A. Manaker
Dr. J.B. Moloney
Dr. L.R. Sibal
Mr. Robert Velthuis

Administrative Staff, Office of the Associate Director, VOP

Mr. John Miller, Administrative Officer, DCCP
Mr. N. Olimpio, Program Analyst, DCCP
Mr. R. Velthuis, Administrative Officer, VOP
Mr. R. Hendricks, Administrative Assistant, VOP

3. Administrative Highlights

The FY 1977 funding level for the Viral Oncology Program was approximately the same as the two previous fiscal years. However, there was again this year some reprogramming of funds within the Program. The amount allocated for Cancer Research Emphasis Grants (CREG) was increased to $1.8M. An additional $0.7M was allocated to the Intramural Research Program. Year-end funds were received from DCCP which did not accrue to the Program base. A funding summary for fiscal years 1976 and 1977 is provided below (in thousands):

	FY 1976	FY 1977
Virus Cancer Program	23,141	24,184
Research Support Contracts ...	11,614	9,673
FCRC	11,361	7,262
CREG	752	1,800
Intramural (including support contracts)	16,615	17,328
TOTALS	63,483	60,247

No new personnel positions were allocated to the Program; however, the transfer of Dr. Katherine Sanford's section from the Division of Cancer Biology and Diagnosis to the VOP increased personnel strength by 10 for a total of 234. Of the 100 Cancer Experts available to the Institute, the Program supported fifteen.

Construction of new laboratories in the Open Bay Area of Building 41 was completed at the end of the fiscal year. Occupancy of the laboratories by the Laboratory of Tumor Virus-Genetics is planned for early FY 1978.

The VOP responded favorably to a request by NIH for use of one of the Building 41 containment suites for DNA recombinant research. Suite 300 was made available. The present occupants of the Suite will be relocated to Building 37 in FY 1978. In exchange for the use of Suite 300, NIH and NCI agreed that two VOP on-site project officers could remain in leased space at Meloy Labs and Hazleton Labs until suitable space became available on the NIH campus at some future time.

4. Scientific Activities

a. Narrative

Introduction

The exciting developments in viral oncology during the last decade have led to a re-shaping of some of our "traditional" views of the viral etiology of cancer. Major emphasis is no longer placed upon the search for a human cancer virus in the form of a discrete, infectious entity which causes malignancy in the same predictable fashion as the cancer viruses of some lower animals. Instead, tumor viruses per se are increasingly becoming regarded as tools for deciphering the molecular pathways by which a normal cell is transformed into a malignant one. Virologists are now concentrating upon looking in normal and malignant cells for pieces of viral genetic information which may code for the proteins that trigger malignancy, and sophisticated efforts are underway to isolate and analyze these gene products. The distinction between virus and cell as separate entities which are mutually exclusive except during the disease process is being replaced by the realization that seemingly normal cells may contain genetic information placed within them by a virus many cell generations before. Indeed, such information may have been placed in the cells of the species' evolutionary progenitors during previous geological periods. Stress placed upon the cell by hormones, the aging process, radiation, certain chemicals, and other influences may activate this dormant information, resulting in the synthesis of a few virus-directed proteins or possibly even an entire virus particle. Among these proteins might be those responsible for activating the malignant process.

Despite the enormous scientific advances made in recent years, the precise mechanism by which a cell becomes malignant remains an enigma. However, there is reason to believe that viruses or viral genetic information may be involved in most malignancies. Where appropriate detection systems have been developed, the carcinogenic activity of chemical and physical agents has often been found to accompany the activation of a latent viral genome. Moreover, even the presence of a whole viral genome is not necessary for a malignant conversion; in all tumor viruses studied, the transforming information is contained in one gene or a small number of genes that can be expressed in the absence of other viral functions.

RNA Viruses

The techniques of nucleic acid hybridization have permitted analysis of the integration of viral information into the host cell genome and the subsequent cellular control over the expression of that information. With suitable probes, the presence of virus information can be determined in the absence of virion production. With suitable diagnostic reagents and large quantities of viruses, the translational products, viral protein components, can be studied. During the past year a great deal of information has been amassed to help understand processes of malignant disease and to develop rational approaches to prevention of human cancer.

Type C Viruses. While some RNA tumor viruses exist primarily as infectious particles, most are in a repressed form integrated into the cellular chromosomes. These endogenous viruses are derepressed by x-irradiation, chemicals, and infection by other viruses. However, some endogenous viruses replicate very poorly in the cells that harbor them (xenotropic) and require special techniques of cell fusion of growth in a mixed culture. Often they can productively infect only cells from animals foreign to the host species. Another class of endogenous viruses (ecotropic) preferentially infect and grow in cells from their own host species. There are also endogenous viruses which are defective for replication and can only be recognized by the presence of their proteins or nucleic acids.

Related RNA viruses readily undergo genetic recombination in cells. Such exchanges occur between endogenous and infectious viruses of the same species and between viruses of different species. Interference between xenotropic and ecotropic viruses does not occur; interference does exist among members of the same class of viruses. Thus, replication of xeno- and ecotropic viruses in the same cell can result in phenotypic alterations involving the antigenic coat and host range of the progeny viruses. Recent studies show that genetically stable recombinant viruses also occur. Such recombinants may be important as the real oncogenic variants, since highly oncogenic strains of sarcoma and leukemia viruses have been observed as a result of genetic recombination. The rapidly transforming virus, the Friend spleen focus-forming virus (a defective virus), is an example of a recombinant between an eco- and xenotropic mouse type C virus. Some of these type C viruses readily transform cells in culture and most produce cancer in animals in days rather than months after infection. These highly oncogenic viruses contain two types of genes: those involved with viral replication and virion structural proteins, and those specifically responsible for cell transformation.

Recently, it has become possible to separate the transformation gene sequences from the replication gene sequences. Essentially this is done by breaking DNA transcripts of viral RNA into pieces and absorbing out those pieces that have replication sequences by a series of nucleic acid hybridizations, leaving only the transformation sequences. Thus, preparing specific cDNA probes from mixtures of sarcoma and helper viruses permitted selecting of virus mixtures highly enriched for sarcoma virus-specific RNA. K-MSV and H-MSV strains have been found to contain a unique set of rat sequences which have become associated in covalent linkage to MuLV information. These rat sequences, called 'sarc' genes, apparently code for the transforming functions of sarcoma viruses.

The transforming sequences from different highly oncogenic viruses have been purified and it has been reported that these transforming sequences are widespread in the normal cells of the animals from which they are derived. However, these sequences represent only a small part of the complete viral RNA and apparently form no protein product that is contained in the viral particle. Consequently, until the transforming sequences were purified, the probes that were used to detect viral activity could not detect either transforming sequences or their products. With the new transformation-specific probes it is now possible to detect viral activity where none could

be found with conventional probes. By this means it has been shown that the transformation sequences are activated both in naturally-occurring and in chemically-induced rat tumors that show no other evidence of viral activity. The transformation sequences thus represent important evidence for a common step in the mechanism of oncogenesis by agents as different as viruses and chemical carcinogens.

The transforming sequences studied so far are from rodent and avian species. Much more important is the fact that a cDNA probe for the sarcoma specific sequences of a primate sarcoma virus, woolly monkey sarcoma virus, has been prepared. These transforming sequences may be sufficiently similar to human transforming genes to be used as probes for the presence of such sequences in man.

Not all transforming sequences from one species of animal are the same. For example, the sarcoma ('sarc') sequences and leukemia ('leuk') sequences of rodents are not related. It is likely, therefore, that different genetic sequences exist for each form of cancer. Although the sequences are different, they may be producing transforming proteins with very similar functions in the cell.

Following the isolation of the rat 'sarc' gene sequences, an exciting recent development has been the in vitro synthesis of at least one protein specified by the 'sarc'-specific region (MSV). This translational product is a sarcoma-specific protein of m.w. 21,000 daltons. Its identification represents a major achievement toward ultimately identifying the cause of cancer since it will be possible now to search for a completely new class of proteins that may actually be responsible for the malignant transformation of cells.

The molecular control of virus expression is receiving intensive study. In particular, the regulation of chromatin transcription should shed light on the control of viral transcription. From genetic experiments in mice we know that the expression of endogenous viral genes can be controlled independently of each other. Thus, in some strains the envelope gene may be turned on but no whole virus produced. Some chemicals can selctively induce a single virus type in mouse cells containing three different endogenous viruses. Since we now know that expression of the transforming sequences of the virus alone can cause tumor formation, it is extremely important for an understanding of malignant disease to know how individual genes are derepressed.

The genome of mammalian type C viruses includes sequences coding for an envelope glycoprotein (gp70), env gene, the viral reverse transcriptase, pol gene, and a gene designated gag which codes for several viral structural proteins. Virion proteins coded for within the gag gene are synthesized in the form of a 65,000 dalton precursor polypeptide which is subsequently cleaved to proteins of 30,000 (p30), 15,000 (p15), 12,000 (p12) and 10,000 (p10) daltons. An analysis of viral antigen expression in cells infected with MuLV mutants defective in precursor isolates led to the identification of several intermediate cleavage products. By this means the gag gene sequence was deduced to be 5'-p15-p12-p30-p10-3'. The structural protein, p10, was shown to be virus-coded and to possess strong group-specific antigenic determinants. By use of appropriate immunoassays, broadly reactive interspecies determinants shared by mammalian

type C virus isolates of murine, feline, and primate origin, were also demonstrated. The interspecies antigenic determinants of p10 were shown to be as broadly cross-reactive as those exhibited by the major type C virus structural polypeptide, p30.

Several low molecular weight proteins of endogenous viruses of the RD114/baboon group were compared with the gag gene-coded translational products of endogenous type C viruses of murine origin. The p10 proteins of each virus group are immunologically and biochemically related, while the p12 proteins of RD114/baboon viruses were demonstrated to share antigenic determinants with murine viral p15. Morevoer, highly type-specific phosphoproteins, p15 of RD114/baboon viruses and p12 of murine viruses, possess analogous biochemical properties. These findings, along with previous studies indicating immunologic cross-reactivity between their major internal antigen, p30, demonstrate that each of the gag gene-coded proteins of murine type C viruses has an analogue in viruses of the RD114/baboon group. The immunologic and biochemical relatedness of their gag gene translational products supports the concept of a common progenitor in the evolution of these endogenous viruses.

Type C RNA viruses have been shown to exist as endogenous viruses within the cellular genomes of many mammalian species. A major research effort is aimed at devising immunological approaches for detection of endogenous virus expression in human tissues. Techniques have been successfully developed for the detection and partial purification of several type C virus-coded proteins from virus-negative cells of a variety of species. Efforts have focused on the search for viral antigens in primate tissues. It has been possible to show that species representing each of the major genera of Old World primates express antigens related to the major structural proteins of the baboon-RD114 virus group. These antigens were shown to have diverged sufficiently so as to be immunologically distinguishable. Differences in the levels of expression of endogenous virus-coded proteins in tissues of a variety of primate species suggest differences in cellular regulation of virus expression by these species. With highly sensitive immunological techniques now available, analogous approaches are being utilized in the search for evidence of endogenous virus expression in higher apes and man.

The biological functions of gp70 (env gene) were studied: gp70 itself was found to interfere, hemagglutinate, and induce neutralizing antibody. Cell attachment, interference, and the neutralizing antigen site were all on the protein portion of the molecule; carbohydrate was necessary only for hemagglutination. The analysis of surface glycoprotein molecules of RNA tumor viruses with regard to specific antigenic and biological functions stimulated the question of whether protection from leukemia could be achieved by active immunization. The finding of interspecies reactive determinants on gp70 molecules allows the possibility that other species may be protected even if the causative virus was not isolated. Recently, the presence of RNA tumor virus envelope glycoproteins on the surface of normal cells has received considerable attention. This interest relates to the larger question of whether the expression of endogenous viral antigens have a functional role in cellular differentiation. The most detailed studies of tumor virus glycoproteins (gp70) have been done in inbred strains of mice. Virion-

associated gp70 molecules exhibit considerable diversity, possibly arising from recombination, in spite of the fact that they share the common property of facilitating entry into mouse cells. Nevertheless, gp70 molecules can be divided into groups related to viral host range classes.

The possibility of using vaccination procedures to control virus-induced tumors has gained renewed interest. A number of laboratories are developing viral vaccines to prevent natural or induced cancer in animals. These experiments are of necessity long-term endeavors because animals have to be followed throughout their lives for definitive results. Encouraging results have been obtained in experiments on spontaneous tumors in cats and mice. For example, cats infected later in life with FeLV are protected from disease by their own antibody response. Similarly, newborn kittens immunized before viral challenge can also be protected with a vaccine prepared from feline leukemia virus (FeLV) or from the membranes of virus-infected cells. An antigen on FeLV-infected cells (FOCMA) appears to elicit an antibody response that decreases tumor incidence. The antibody to FOCMA is independent of antibody to either of the major FeLV structural proteins p30 or gp70 and argues against the possibility that FOCMA is a virus-coded structural protein. Sarcomas can be presented in cats by passive immunization with antibodies directed against viral envelope antigens of FeSV; existing tumors also regress following this treatment. In mice, similar passive protection against Friend disease was obtained by the administration of antiserum to gp70. A significant reduction of virus titer has been achieved by vaccination of mice with combined killed and live type C virus vaccines. Previous studies have shown a close correlation between virus titer and cancer incidence in the strains of mice used in the vaccine experiments.

Type B Viruses. Experimentation requiring large quantities of MMTV has been limited by the difficulties inherent in recovery of virus from mouse milk. This virus has now been propagated successfully and harvested in quantity from long-term cultures of BALB/cf C3H mouse mammary tumors. The virus was also shown to replicate in cultured mink lung cells and cat kidney cells. difference was detected between the RNA of MMTV grown in mink cells and that recovered from the milk of mice. These advances permit more intensive molecular biological studies on mammary gland carcinogenesis than have been possible previously.

The sequences of the RNA genomes of the MMTVs from C3H and RIII mice that are transmitted via a non-germ line mechanism have been isolated. These sequences are present as provirus in the DNAs of mammary tumors of C3H, RIII, and GR mice but are not detected in mammary tumors of C3HfC37BL or BALB/c mice. These sequences are also not detected in the DNA of apparently normal tissues of several strains of mice. Independent polypeptide initiation sites have been demonstrated for the MMTV proteins (p28, p22, p18, p14, and p10).

Hormones also play an important role in virus expression. This has been clearly demonstrated for the type B RNA viruses, the growth of which is greatly stimulated by dexamethasone. Mammary epithelial cells, the target cells in which type B viruses grow, also are subject to hormonal control. This may

reflect a significant relationship between cell activity and viral growth. Thus, the factors governing mammary tumorigenesis are highly complex but are closely correlated with factors which regulate the expression of mammary tumor virus expression.

The free glycoprotein of MMTV was detected in the circulating blood of tumor-bearing mice in concentrations that correlate with tumor load. In the strains used for this study, each gram of tumor led to the production of 500 ng of glycoprotein; blood levels dropped dramatically within 8 days after the tumor was surgically removed. Subsequent assays for the antigen predicted relapse in the animals with complete accuracy. This model for cancer diagnosis and prognosis was recently applied to studies on breast cancer patients. Plasma levels of the reverse transcriptase from MPMV were found to correlate with the state of this disease.

The Mason-Pfizer monkey virus (MPMV) was initially isolated from a breast tumor of a rhesus monkey. Subsequent isolates have also been obtained from normal rhesus monkeys. MPMV differs in both its structure and the antigenic determinants of its major structural protein from those of any known RNA tumor virus. However, MPMV, like mammalian type C viruses, contains a glycoprotein whose molecular weight is approximately 70,000. An interspecies immunoassay utilizing antiserum to MPMV to precipitate baboon viral gp70 has been shown to detect antigenic determinants shared by glycoproteins of MPMV, RD114, and baboon type C viruses. These findings indicate that MPMV may have originated by recombination with endogenous primate type C viral genetic sequences.

New Viruses. Two isolates of type C viruses were obtained from Mus cervicolor. One of these is immunologically related to the woolly/gibbon group of infectious type C viruses. The other isolate shares antigenic determinants with type C virus of M. musculus. In addition, a new class of endogenous virus was isolated from Mus cervicolor and Mus caroli. These viruses are morphologically distinct from all other RNA tumor virus classes and are unrelated by immunologic and nucleic acid homology to all murine type B and C viruses.

Different viruses have been isolated from various primate species by co-cultivation of tissues with heterologous cells. These isolates include type C viruses from several species of baboons (Papio) and from the closely related genus, Theropithecus. In addition, a virus has been isolated from a langur monkey cell line. This isolate is related to MPMV, but can be readily distinguished from MPMV by its host range, antigenic properties, and by nucleic acid hybridization. Various isolates from squirrel monkey cell lines have also been obtained which are endogenous to these monkeys, and are present in multiple copies in the cellular DNA of all squirrel monkey cells and tissues.

DNA Viruses

The role of DNA viruses as etiologic agents of malignant disease has now become an accepted fact. Polyoma virus, SV40, and the papilloma viruses, members of the papova group of viruses, not only produce tumors in several different animals but also transform cells in tissue culture. These early experiments which demonstrated the oncogenic properties of murine and simian papovaviruses provided the stimulus for examining the relationship between various human DNA viruses and cancer in man.

The first class of viruses considered in this context were the human adenoviruses which have been shown to act as biological carcinogens in selected rodent systems. Prior to this demonstration, various live, attenuated adenovirus vaccines were administered to selected human populations as a control measure for debilitating respiratory tract infections. A further complication was introduced when it was discovered that the oncogenic papovavirus SV40, acquired from the simian cells used for propagation of the adenoviruses, was present as a major contaminant in these vaccine preparations. Since hybrid viruses with a spectrum of biological functions have been isolated from mixed adenovirus-SV40 populations, these adenovirus vaccines undoubtedly contained such recombinant viruses. Thus, more than one million people were inoculated with representative members of two groups of DNA viruses with known oncogenic properties.

The possibility that members of this group might be involved in human cancer was investigated by screening sera from cancer patients for antibody to the T-antigens induced by these viruses in transformed cells. No significant patterns of serological reactivity emerged from such studies. Hundreds of human tumor specimens were then examined for adenovirus-specific-mRNA and for viral DNA using probes that could detect each of 31 types of adenovirus, but again no evidence of viral information was found in these specimens. The continued study of a possible association between these viruses and human cancer still seems relevant because: (1) these agents produce malignant tumors in several species of animals and morphologically transform a variety of cultured cells, including those of human origin; (2) human adenoviruses are widely distributed and interact genetically with the oncogenic simian virus, SV40, inadvertently administered to a large group of people as a vaccine contaminant; (3) several human papovaviruses have been found to be widely distributed in human populations; (4) genetic information homologous to one of these agents (BK virus) has been detected in several human tumors; and (5) these small DNA viruses offer useful models for studying the mechanisms underlying the process of cell transformation.

The papovaviruses have provided a particularly fruitful approach for studying the phenomenon of transformation of a cell from the normal to the malignant state. These small, relatively simple viruses perform the convenient function of packa all of the genetic information required for the induction of cancer. The fact that the DNA of these viruses codes for the synthesis of less than 10 proteins makes them especially attractive in terms of defining e virus-specific functions which are responsible for oncogenesis. Indeed, significant progress has already been made along these lines. Cleavage of the

viral DNA with restriction endonucleases has led to the generation of probes representative of specific portions of the viral genome. These probes have been used to identify the transforming genetic sequences of SV40 and certain adenoviruses and to map the cellular integration sites of SV40 in transformed cells. Analysis of cells transformed by mutants of SV40 has led to the identification of a virus-coded protein (A protein) which is required to initiate and maintain cell transformation. This early viral protein, related immunologically to the intranuclear tumor antigen (T antigen) found in all SV40-transformed cells, is also required for the initiation of viral DNA synthesis. The T antigen has now been extracted from transformed cells and experiments have been initiated to define the molecular interaction between this purified protein and cellular genetic information during the transformation process.

Herpesviruses recovered from frogs, chickens, rabbits, and non-human primates are known to be causally related to carcinomas or lymphoproliferative neoplasms in their respective hosts. For example, two simian herpesviruses cause malignant T-cell lymphomas in animals: Herpesvirus saimiri, recovered from squirrel monkeys, and Herpesvirus ateles, isolated from spider monkeys. Some owl monkeys infected by these viruses develop a malignant leucoproliferative disease. Both viruses induce malginant lymphoma in rabbits. The cotton-top marmoset is susceptible to H. saimiri-induced lymphoma, but Cebus monkeys and Macaque species were not affected.

The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma stimulated extensive studies to examine herpesviruses as possible etiologic agents of human cancer. There is now extensive immunological, biological, and biochemical evidence which supports a determinative role for EBV in lymphoma and nasopharyngeal carcinoma (NPC) and for herpes simplex virus (HSV) type 2 in squamous cell carcinoma of the uterine cervix. Although a causal relationship has not been definitively established, most of the experimental and epidemiological data strongly suggest the involvement of these viruses with malignancy in human carriers.

Both EBV and HSV share several properties normally associated with DNA viruses with known oncogenic potential. These two human herpesviruses transform cells in vitro and are found as infectious virus or virus-specific information in each of their associated tumors. In addition, fatal, malignant diseases have been induced in appropriate hosts following inoculation of virus or virus-transformed cells. Cell transformation seems to result from an abortive cycle of virus replication in which viral functions responsible for inhibition of host cell macromolecular synthesis are not expressed.

A key problem encountered in assigning an etiologic role for herpesviruses in human cancer has been their wide distribution in human populations. Tumor formation is usually marked by an increased, rather than a de novo, immunological response to the virus, although antibody titers in cancer patients are much higher than those levels measured in carefully matched normal subjects. Patient immune status to EBV-related antigens correlates with the risk of primary Burkitt's lymphoma or relapse following therapy and with the clinical status of nasopharyngeal carcinoma. The question of why some individuals infected

with these viruses develop cancer while others do not remain unanswered. It appears that EBV expression is controlled at the host level and disseminated following immune impairment. The repressive functions associated with the cell on one hand and the normal host on the other hand might be factors controlling the development of overt lymphomas. It is entirely possible that these viruses act in concert with other biological, environmental, or genetic factors to initiate the neoplastic process. The possible cocarcinogenic activity of human herpesviruses is presently being investigated not only in the United States but in other epidemiologically relevant areas throughout the world.

In the laboratory, experiments are in progress to isolate and characterize herpesvirus-specific nucleic acids and antigens which can be employed as diagnostic or prognostic indicators of tumor development. Attempts are being made to define those regions of the herpesvirus genome which code for cell transformation, to characterize the proteins specified by this genetic information, and to study the interaction of these viral components with normal and malignant cells. The methodology employed in these studies largely reflects that used by tumor virologists studying cell transformation by the papova- and adenoviruses. These include dissection and characterization of the viral DNA with restriction endonucleases and the genetic analysis of mutant viruses which have lost the ability to transform cells in vitro. Progress in this area has been impeded by difficulties encountered in working with such a genetically complex virus. The herpesvirus genome, with a molecular weight of 100×10^6 daltons, is 5 and 25 times larger than the genome of the adeno- and papovaviruses, respectively. Nevertheless, a number of Herpes simplex virus proteins and glycoproteins have been isolated and purified. Among these, VP123 possesses antigenic determinants predominantly specific for HSV-1 and VP-119 for HSV-2. New experimental approaches and methodologies are being developed to deal with these problems.

If the herpesviruses are shown to play an etiologic role in human cancer, specific measures can be developed to control their transmission and expression and thereby reduce the incidence of human neoplasia. If these viruses do not act as primary carcinogens, continued study appears warranted based on their role as cocarcinogens and on their utility as specific prognostic and diagnostic aids for certain malignant diseases.

b. Progress Highlights

RNA Viruses

The availability of larger amounts of MMTV from cell cultures than possible from mouse milk and of antisera to purified virion polypeptides has provided increased opportunity to study the disease process in mice and to probe for related components expressed in mammary cancers in humans and in other animals.

A single dominant gene (Mtv-1) in the C3Hf mouse strain controls the release into milk of MMTV-L, the low oncogenic variant of mouse mammary tumor virus. Mtv-2, which is responsible for release of high levels MMTV-P and early hormones dependent mammary tumors, consists of about eight tandem copies of MMTV-DNA.

Vaccination of mice with gp52, a glycoprotein component of MMTV, resulted in a slight delay of mammary tumor development. Resistance to tumor transplants was obtained when low doses of vaccine were used with an adjuvant; higher doses of MMTV protein accelerated tumor growth and appeared to be associated with poor cellular immune reactivity to the virus.

Antisera to the major glycoprotein component of MMTV, gp52, were used to probe for related antigenic determinants in human milk specimens fractionated by zonal centrifugation. Some positive findings were obtained with pooled milks, but the negative results with individual specimens suggested very low concentrations or infrequent occurrence of the reactive component.

The detection in tumor tissue of reverse transcriptase (RT) activity suggests the presence of RNA tumor virus genetic information. Extracts of 20 human placentas examined contained RT activity. Electron microscope studies detected virus-like particles in the trophoblast layer of 7 of 19 human placentas. The enzyme activity was not neutralized by antisera against the RT of the known primate, feline, or murine type C viruses. The virus has not yet been propagated in cell cultures.

Human leukemic cells were probed for the presence of antigens related to simian sarcoma virus (SSV-1), previously isolated from a woolly monkey. No evidence of antigens related to the p30 or gp45 virus polypeptides was found. However, nucleic acid sequences related to the virus nucleic acid were reported to be present in specimens from some patients with acute myelogenous leukemia. A glycoprotein with m.w. of 55,000 daltons detected in membranes of human chronic granulocytic leukemia cells appears to be related to a minor antigenic determinant on the gp71 of the Friend murine leukemia virus.

Considerable work has been done to characterize the protein products of RNA tumor virus genetic expression. Many gp70 MuLV envelope glycoproteins were isolated from viruses purified from sera and excretions of several classes of related virus envelope proteins. More than one type of gp70 could be isolated from a single mouse strain, depending on the anatomical site or origin. Thus, virus-specific cell surface antigens have been identified which may be important in identifying preleukemic cells and in immunoprophylaxis.

Studies on precursor proteins were conducted to define the gene order in oncogenic type C viruses. The structural (gag) gene is adjacent to the polymerase (pol) gene in the virus genome. The control of virus gene expression in mice was linked to the differentiation of lymphoid and epithelial cells in some mouse strains.

Mice may develop a natural immune response to antigens of their endogenous viruses depending on the expression of the antigen in tissues and on host genetic factors. The effectiveness of active immunization of mice with purified viral gp71 envelope protein varied among mouse strains. A protective effect was observed in mice of low leukemia strains. Mice of high leukemia strains suffered enhancement of spontaneous disease following active immunization, but this was circumvented by passive immunization with heterologous anti-gp71 serum. Passive immunization with antisera against the interspecies antigens of leukemia viruses also had a protective effect against virus challenge.

Recently, a type C virus, not yet completely characterized, was recovered from a human histiocytic lymphoma cell culture. This virus is related but not identical to the simian sarcoma virus.

Host immune responsiveness to virus-associated or tumor cell-associated antigens holds promise as a tool in diagnosis or prognosis for some cancers.

Appropriate model systems have been developed to identify a protein responsible for the transformation of a normal cell to a malignant one. In a cell transformed by an RNA tumor virus, a protein (m.w. 21,000 daltons) which appears to be necessary for the maintenance of the transformed state, is produced. This observation implies (a) that continued expression of a viral gene is necessary for the maintenance of transformation and (b) the cancer process itself is potentially reversible. Using a system involving KMSV and HMSV, a unique set of rat genetic information responsible for peroblast transformation and proteins coded for by the rat sequences have been identified. Additionally, some of these endogenous viruses are replication-defective suggesting that the human RNA tumor viruses may also be defective. Very different approaches may be required for identifying human viruses.

Since human neoplasia is apparently not associated with a replicating type-C virus, the rat system offers an excellent model system for establishing viral etiology of human cancer. The system not only permits the testing of various chemical carcinogens but distinguishes between potential and actual chemical carcinogens.

The defective component of the Friend virus has been isolated for the first time. It has been shown to represent a recombinant between an ecotropic mouse type-C virus and a xenotropic mouse type-C virus. The finding that the rapidly transforming virus, the Friend spleen focus-forming virus, has acquired endogenous, xenotropic viral information reopens the question of the role of these viruses in the etiology of leukemia in both rodent and primate species. Since endogenous viruses have been found in Old World primates and in New World species of lower vertebrates, the likelihood that replication-defective, viral

information may be responsible for both solid tumors and leukemias in humans is very high.

The expression of the mouse type-B viruses, which induce mouse mammary tumors, can be stimulated by a glucocorticoid hormone, dexamethasone. In the past year, the physiology of the mechanism of action of this glucocorticoid hormone has been elucidated. It stimulates the rate of transcription of MMTV-RNA in the absence of new protein or DNA synthesis. This finding permits developing new approaches to the use of hormones in treating human cancer.

A step in the viral replication cycle which involves either the circularization or integration of viral DNA into host genetic sequences has been identified as the locus of action of the viral target molecules. Future elucidation of the mechanism of action of these molecules could lead to ways of preventing the replication of type-C virus at novel steps in their replication pathway, and thus be useful in the prevention of viral-induced leukemogenesis.

The "sarc" sequences in mouse cell DNA are highly conserved. In contrast the "common" sequences are not conserved and no related sequences can be found in a closely related mouse species. Different portions of MSV appear to have a distinct history, supporting the theory of a recent recombination event producing MSV. The "sarc" sequences may have some important normal cellular function because they are so highly conserved.

Cloned mouse cells which reverted from the MSV transformed state have no expression or MSV specific RNA. These cells are not rescuable for MSV after MuLV infection of induction by halogenated pyrimidines. Some revertant clones back-transform spontaneously; some of these expressed MSV-specific-RNA indicating that the MSV genome was present during the revertant phase.

The subunits of RNA from mammalian sarcoma viruses were found to be 28S to 30S in contrast to 30S to 40S RNA subunits for leukemia viruses. The 3'-terminal of viral RNA subunits from mammalian leukemia and sarcoma viruses contained poly(A)-rich sequences, and poly(A)-, poly(C), and poly(G)-rich sequences were present in the RNA of leukemia and sarcoma viruses; however, poly(U)-rich tracts were absent in the viral RNA.

Hybrid viruses composed of eco- and xenotropic oncornavirus information have been isolated; these are recombinants in the major glycoprotein region which is located near the 3' end of the RNA molecules. To determine whether such recombinant viruses with a broad host range play an active role in oncogenesis, cloned variants were inoculated into mice and cats. The recombinant was able to induce lymphomas rapidly in several strains of mice but not in cats.

All the mammalian and avian type C viruses were found to contain a major structural phosphoprotein which specifically binds to the homologous viral RNA genome. Assays have been developed to study the specificity of such protein-RNA interaction in the different classes of type C viruses. The close association with the viral genome, the ability of specific interaction with homologous viral RNA and the covalent modification (phosphorylation) suggests a possible regulatory role of the type C viral phosphoprotein in host-virus interaction.

The relationship between sarcoma virus transformation and the expression of available membrane receptors for several polypeptide ligands were examined. Transformation of cells with either murine or feline sarcoma viruses specifically abolished the binding of epidermal growth factor (EGF-a polypeptide growth factor) to its cellular membrane receptors.

A new isolate was obtained from a Southeast Asian primate species, the langur. This virus, designated PO-1-Lu, is morphologically and biochemically related to a virus isolated from rhesus monkeys (Mason-Pfizer monkey virus). PO-1-Lu and MPMV can be distinguished from one another by immunological and nucleic acid hybridization criteria. The isolation of a virus related to MPMV from a second primate species whose natural habitat overlaps that of rhesus monkeys suggests that this group of RT-containing viruses may be endemic to Southeast Asian primates.

Several new virus isolates have been obtained from the Southeast Asian murine species Mus caroli and Mus cervicolor. The first subgroup is immunologically related to the woolly monkey type C virus and a type C virus isolated from Mus caroli. The second is immunologically related to but can be distinguished from standard mouse viruses. The Southeast Asian mouse viruses contain a 70S RNA genome which can be dissociated into 30S subunits. There are at least six virion associated proteins which are presumed to be encoded by the viral genome; these include the reverse transcriptase (Mg dependent), two envelope proteins (gp65 and gp32), and three internal structural proteins (p24,p16,p12).

Mouse mammary tumor viruses (MMTVs) from three different strains of mice have been employed to establish productive infections in feline and mink cell lines. The virions being released by these cells compete completely in a radioimmunoassay for the major virion surface glycoprotein in MMTV (gp52), thus demonstrating that antigenic determinants of gp52 are viral coded. Competitive molecular hybridization studies have shown that the 60-70S RNAs of MMTVs propagated in feline cells contain all the nucleic acid sequences found in 60-70S RNA from MMTV synthesized by murine cells.

The development of immunologic approaches for study of different proteins of mammalian type C viruses has led to the establishment of immunoassays for each of the known viral-coded proteins. These included p30, p15, p12, and p10, each of which is coded as part of a polyprotein precursor of the gene, designated gag. The viral reverse transcriptase (pol gene) and the envelope glycoprotein, gp70 (env gene) have all been characterized physically and by radioimmunoassay.

Interspecies antigenic determinants are shared between the analogous p30s, p10s, p15s, and reverse transcriptases, respectively, of all known mammalian type C viruses. These findings strongly argue for a common progenitor in the evolution of mammalian type C viruses.

In studies designed to elucidate the structure of the type C viral genome, the order of the gag gene of mammalian type C viruses has been found to be 5'-p15-p12-p30-p10-3'.

The gag polypeptide p60, specified by the defective Moloney murine sarcoma virus genome, has been extensively characterized. Antibody prepared to the purified protein was shown to recognize determinants on Moloney murine leukemia virus (M-MuLV) p30, p15, and p12. In complement-dependent assays the anti-p60 serum was shown to neutralize heterologous RNA tumor viruses and to cause virolysis.

Efforts have focused on the search for viral antigens in primate tissues. It has been possible to show that species representing each of the major genera of Old World primates express antigens related to the major structural proteins of the baboon-RD114 endogenous virus group.

DNA Viruses

Members of the papovavirus group of small DNA viruses have been shown to be oncogenic in laboratory animals. Two members of this group, simian virus 40 and the BK virus isolated from humans, are under study. Nucleic acid hybridization analyses, using viral DNA probes, were applied to search for the presence of viral genetic information within human tumors and in cultured tumor cells. To date, no evidence of the presence of BK or SV40 copies were detected in these tissues.

Patient immune status to EBV-related antigens correlates with the risk of primary Burkitt's lymphoma or relapse following therapy, and with the clinical status of nasopharyngeal carcinoma. Cell immune reactivity to EBV antigens also correlates with disease status in some cases.

The influence of the host and of the virus-carrying cell on the virus-cell relationship was demonstrated by implantation into nude mice of the EBV-carrying HR1-BL cells and of somatic cell hybrids of HR1 with D98 cells. Both the HR1 and the HR1/D98 hybrid cells grew to produce tumors in these mice. A considerable reduction in the amount of viral DNA carried by these tumor cells was observed following their passage in vivo. Furthermore, the HR1 cell tumors from nude mice were observed to contain a type C RNA virus budding from cell membranes, apparently a xenotropic virus activated in the nude mice bearing the tumor and replicating in the tumor cells.

A number of HSV-induced proteins and glycoproteins were isolated and purified. Among these, VP123 possessed antigenic determinants predominantly specific for HSV-1 and VP119 for HSV-2. By radioimmune precipitation tests, type-specific antibody could be detected in the sera of humans with primary HSV-1 or HSV-2 infections.

Two simian herpesviruses cause malignant T-cell lymphomas in animals: HVS, recovered from squirrel monkeys, and HVA, isolated from spider monkeys. Some owl monkeys infected by these viruses develop a malignant leucoproliferative disease. Both viruses induce malignant lymphoma in rabbits. The cotton-top marmoset is susceptible to HVS-induced lymphoma, but Cebus monkeys and Macaque species were not affected.

An intensive investigation was made to determine whether an association exists between adenoviruses and human cancer. These viruses induce tumors in hamsters. Homology between the DNAs of adenoviruses of different groups showed that the analysis of tumors with DNA probes prepared from 6 or 7 virus types could determine the presence of DNA for 31 types. An analysis of human tumors was made with adenovirus-transforming genes; the study included lung, colorectal, bladder, and pancreatic tumors, but no virus DNA was detected.

The T-antigen in SV40-induced tumors and in lytically infected cells is coded by about one-half of the virus genome corresponding to the early region as defined by transcriptional mapping. The other half of the genome, the late region, codes for viral structural proteins.

Immunology

Immunoprevention against leukemias which reach incidences of 90 to 100 percent in AKR mice at a year of age was achieved with the use of virus-specific immunoglobulin (IgG) prepared in goats against the AKR and other ecotropic viruses. The use of combined killed and live vaccines given at critical time periods in several AKR crossbreeds have thus far provided significant protection against leukemia.

Rat tumor cells, including those transformed spontaneously and those transformed by DNA and RNA tumor viruses, were found to have a common tumor antigen when tested by antisera from rats carrying these various tumors. The common tumor antigen does not crossreact and presumably is not related to the rat type C virogene.

Protection against transplantation of lethal doses of rat tumor cells was accomplished by preimmunizing the rats with 10^6 to 10^7 KiMSV(NP) transplantable tumor cells. Although the KiMSV(NP) cells replicate subcutaneously, the tumor was completely rejected within 2-3 weeks, following which a powerful cell-mediated immunity prevented the establishment of other highly lethal rat tumor cell transplants.

Active immunity to radiation-induced leukemias in C57BL mice was established by immunization with inactivated R-MuLV and passive immunity by transfer of goat anti-R-MuLV immune IgG. Both regimens resulted in measurable circulating antibody to gp70 throughout part of the latent period of tumor development and both resulted in reduced tumor incidence.

Pyran copolymer was found to be equal to or better than Freund's complete adjuvant when used as an adjuvant with irradiated tumor cell vaccines; thus, it appears to be one of the most potent agents capable of exerting antitumor and immune modulating activity.

The incidence of radiation-induced leukemia in the C57/816 strain can be significantly reduced by passive or active immunization against the mouse type C virus. These findings help to demonstrate that the endogenous viruses are etiologically involved in cancers.

Influenza virus-augmented tumor-associated antigens were successful for preventing metastasis and regrowth of tumors in mice after excision. Infection of the tumor cells with virus apparently stabilizes the tumor-associated antigens against chemical degradation.

Human sera tested by immunoelectronmicroscopy contained natural antibodies to antigens on the envelope of the following type C viruses isolated from primates: gibbon ape leukemia virus, simian sarcoma virus, baboon endogenous virus, putative human virus isolated from a patient with acute myelogenous leukemia (HL23V) and from human embryonic diploid cells. Human sera did not react with normal gibbon spleen monolayer cells but showed positive reactions with nonproducing human embryonic diploid cells which become virus-producing cells after repeated passages.

Seventy-nine percent of 76 sera from healthy persons were positive for VEA of GALV with various antibody activity. A peak of antibody level and incidence of positive sera was observed at ages 20 to 50 years, an observation which corresponds inversely to a leukemia incidence with two peaks at ages 1 to 14 years and again over 50 years.

Specific type C tumor virus antigens are capable of inducing a neutralizing antibody response in the host of origin. The major glycoprotein of MuLV (gp71) was examined and found to possess determinants reactive with the neutralizing antibody. To a lesser degree a smaller surface antigen, the p15E, was also reactive. Type, species, and interspecies determinants on the gp71 molecule could all react with the appropriate virus-neutralizing antibodies.

Analysis of approximately 6,000 sera from randomly selected dairy cattle indicated the presence of antibody against BLV p24 in many of the animals tested, the percentage ranging from a low of 2-4% to a high of 42%. Moreover, sera from a significant number of antibody-negative animals were shown by competition immunoassay to contain high levels of viral antigen. These findings indicate that the infection by this virus may be much more prevalent among domestic cattle than previously believed. Sera from 450 individuals employed in slaughterhouses or on dairy farms as well as over 900 sera from patients with various forms of cancer failed to demonstrate detectable antibody to BLV p24.

It was possible to demonstrate that the antibody to FOCMA was independent of antibody to either of the major FeLV structural proteins, p30 or gp70. These findings argue strongly against the possibility that FOCMA is either of these FeLV-coded structural proteins.

Co-Carcinogenesis

Previous studies showed that cultured murine cells, when infected by a murine leukemia virus, were more sensitive to transformation by chemical carcinogens than were uninfected cells. Other observations showed that several classes of chemical carcinogens, other viruses, and irradiation could activate endogenous virus gene expression in cultured murine cells.

Present data indicate that a single autosomal dominant gene regulates endogenous xenotropic virus expression in mice. The sensitivity of these animals to 3-methylcholanthrene-induced fibrosarcomas was found to correlate with the level of xenotropic virus expression. These animal systems will be used for further study of the interaction between the virus and 3MC and their causal role in tumorigenesis.

Previous studies which demonstrated the ability of UV-HSV to activate type C virus were conducted in BALB/c mouse cells carrying a defective mouse sarcoma virus genome. The activation of the endogenous type C virus by UV-HSV resulted in the release of virions containing the sarcoma virus genome. The data suggest that HSV may act as a co-carcinogen by altering expression of type C virus genetic information.

5. Projections

The Viral Oncology Program has set forth a long range research plan to elucidate the role of viruses in human cancers and to exploit any leads that develop for use in the detection, diagnosis, treatment and prevention of cancer. The studies are grouped within the following approaches:

a. Virus Studies

(1) Basic Studies. Certain basic studies on RNA and DNA viruses in experimental animals and in cell culture will remain an integral part of the Program. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents.

(2) Co-Carcinogenesis Studies. While it appears that many environmental carcinogens (biological, chemical, or physical) induce cancer, there is evidence that such action is mediated by the activation of latent viruses or viral genes. Cell lines in culture and methods suitable for studying viral-viral, viral-chemical, and viral-physical interactions or co-carcinogenesis are now available and such studies will be considerably expanded:

(a) to define the role of endogenous viruses in process of cell transformation;

(b) to increase understanding of the relationship of environmental agents as cofactors in carcinogenesis;

(c) to extend and develop new methods of inducing tumor virus expression in "normal" cells;

(d) to develop reliable, sensitive methods for *in vitro* carcinogen testing.

(3) Human Studies. Attempts to isolate RNA or DNA viruses or to detect the presence of viral genetic information and its expression in human tumors will continue. However, considerable emphasis will be placed on developing new probes that can identify viral transforming activity. These efforts will be applied to human cancers:

(a) to identify and isolate viruses, virus expression or viral gene products in human leukemias, lymphomas, sarcomas, and carcinomas;

(b) to apply specific methods for detecting individuals or groups of individuals at high risk to cancer, i.e., individual susceptibility or predisposition to transformation by viruses.

b. Molecular Studies

Increased effort will be given to molecular studies on the mechanisms of interaction of viruses and cells. Major progress has been achieved in understanding the molecular pathways of tumor virus replication. Such advances have provided the basis for sensitive methods for the detection of oncogenic viruses or virus expression. Tests are now available to characterize information detected in normal and tumor cells from patients with various cancers. Since many tumor viruses may be defective, virion replication may be a relatively rare event in human cells. Therefore, emphasis will be given to developing procedures which can detect events of transformation in the absence of viral replication.

(1) Basic Studies. The Program will intensify its efforts to detect, identify and characterize viral genes ("sarc", "leuk" sequences) and viral gene products. Recent studies indicate that whole virus may not be required for converting a normal cell into a cancer cell, and that only one or two viral genes may be involved. Attempts to isolate a "transforming factor", a protein that triggers cells to become malignant, are nearing successful completion.

(2) Applied Studies. As information about the fundamental molecular events in virus-cell interaction becomes available, it will be applied to the study of human cancer:

(a) to develop new probes and sensitive methods for the detection of virus replication and virus transformation;

(b) to identify and characterize viral genes (nucleic acid sequences) and viral gene products (proteins, antigens) found in normal and malignant cells;

(c) to develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. (The therapy could be directed at any or all of the stages of cell transformation. Ultimately these approaches can be an intensified program to develop anti-enzymes, gene repressors, or other inhibitors effective at the molecular level.)

c. Immunologic Studies

Current trends to concentrate heavily on molecular events in transformation will not detract from the need to pursue immunologic studies. Sensitive, specific methods for the detection of tumor viruses, viral antigens, and tumor antigens found at the cell surface are now available. They are also being applied to understanding the role of immunologic mechanisms in host-tumor and host-virus interactions that may provide a rational approach to the treatment of certain cancers.

(1) Basic Studies. Selected model systems representing tumors induced by RNA and DNA viruses, especially those isolated from non-human primate species, will be studied:

(a) to identify and characterize the viruses, viral antigens and membrane antigens at various stages of the replication and/or transformation process;

(b) to study natural cellular and humoral immune mechanisms in order to determine their relative significance in host recognition of and response to virus-induced tumors;

(c) to develop active and passive immune mechanisms to enhance host response to virus-induced tumors.

(2) Applied Studies. As rapidly as possible, immunological methods will be applied:

(a) to relate putative human viruses or viral information to known oncogenic agents;

(b) to identify and characterize putative human tumor viruses or viral antigens;

(c) to determine the presence of crossreacting antigens suggesting viral causation in various human tumors;

(d) to establish the association of a putative cancer virus with the disease process;

(e) to continue seroepidemiological surveys and case-control studies which will define populations at high risk to cancer, i.e., groups of workers exposed to environmental carcinogens;

(f) to determine the role of host immune responses in virus-induced tumor recognition and rejection;

(g) to develop vaccines (viral or subviral components) from tumor viruses to suppress human cancer.

d. Test Systems and Resources

(1) Test Systems. In vitro and in vivo test systems will be carefully selected to evaluate the work outlined in the previous research areas:

(a) to determine the oncogenic potential of putative human viruses;

(b) to begin viral vaccine (conventional or other) testing and immunization programs;

(c) to explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

(d) to develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species); in particular to develop normal and malignant epithelial cell lines.

(2) Resources. Since research efforts undergo continual change in emphasis and scope as new leads emerge, the Program will continue to develop and maintain a variety of resources:

(a) to collect and store human serum and tissue specimens; integrate data on clinical records, storage and distribution; computerize specimen collection;

(b) to maintain various mammalian animal colonies, especially endangered primate species, for basic research and special studies;

(c) to develop methods to produce candidate human viruses as they are isolated;

(d) to produce large amounts of animal tumor viruses and high quality diagnostic reagents for research;

(e) to provide containment facilities for research on known oncogenic viruses and candidate human tumor viruses; in particular, to provide facilities for conduct of recombinant-DNA studies as appropriate.

6. Reports on Information Exchange Activities, International Agreements, and Meetings Sponsored by the Virus Cancer Program

(Fiscal Year 1977)

International Agreements

US-USSR Agreement on Health Cooperation - Joint Working Group on Cancer Virology

US-France Cooperation in Cancer Research - Committee on Cancer Virology (with emphasis on immunology)

US-Japan Cooperative Cancer Research Program

Meetings Sponsored or Partially Sponsored by the Virus Cancer Program (VCP)

Eleventh Annual Joint Working Conference - VCP (Hershey, PA, November 1976)

Symposium, Guinea Pig L2C Leukemia: Immunological, Virological and Clinical Aspects (Bethesda, MD, November 1976)

Third Conference on Modulation of Host Resistance in the Prevention or Treatment of Induced Neoplasias (Bethesda, MD, December 1976)

Workshop on Tumor Virus Antigens (Bethesda, MD, March 1977)

Workshop on Breast Cancer Virology (Bethesda, MD, March 1977)

International Symposium on Etiology and Control of Nasopharyngeal Carcinoma (Kyoto, Japan, April 1977)

Workshop on Primates and Human Cancer (San Antonio, TX, April 1977)

Third International Conference on Comparative Virology (Quebec, Canada, May 1977)

Third International Symposium on Oncogenesis and Herpesviruses (Cambridge, MA, July 1977)

VIIIth International Symposium on Comparative Research on Leukemia and Related Diseases (Amsterdam, The Netherlands, August 1977)

Meetings to be Sponsored by the VCP

International Symposium on Papovaviruses and Their Role in Cell Transformation and Oncogenesis (Bethesda, MD, Fall 1977)

Fourth International Congress for Virology (The Hague, The Netherlands, September 1978)

Twelfth Annual Joint Working Conference - VCP (Hershey, PA, November 1977)

US-USSR Agreement. A Memorandum of Understanding for further cooperation in the study of the microbiology, the immunology, and the molecular biology of cancer viruses was first signed on November 18, 1972. The Memorandum established procedures for joint studies through the exchange of information, materials and scientists.

Delegation Meetings:	November, 1972 - Moscow, USSR	
	November, 1973 - Bethesda, USA	(Subcommittee)
	May, 1974 - Moscow, USSR	
	May, 1975 - Bethesda, USA	
	June, 1976 - Sukhumi, USSR	
	October, 1977 - Bethesda, MD	

As agreed, the fourth meeting of the US-USSR Joint Working Group on Cancer Virology, Co-Chairmen Dr. J.B. Moloney and Professor V.M. Zhdanov, took place at the Institute of Experimental Pathology and Therapy, USSR Academy of Sciences, Sukhumi, USSR on June 2-5, 1976. At a symposium held on June 3-4, 1976, members of the US and USSR delegations and other participants presented their recent studies on cancer virology. The titles and abstracts of scientific papers presented are published in Russian with English summaries in a monograph of the Academy of Medical Sciences, USSR, titled: МАТЕРИАЛЫ IV СОВЕТСКО АМЕРИКАНСКОГО СОВЕЩАНИЯ ПО ВИТУСОЛОГИИ ОЛУХОЛЕЙ (Materials - Fourth Soviet-American Conference on Tumor Virology). The delegates later discussed the progress of previous collaborative studies, future collaborations and exchange of materials, publications and scientists, noting that progress on the joint research accomplished during the previous year was satisfactory to both Sides.

In particular, the delegates discussed and recognized the importance of scientific collaborations in the following areas: (1) studies of viruses isolated from human tissues in cell cultures or in animals and their possible role in the pathogenesis of human neoplasia; (2) continuation of studies on non-human primate viruses in relation to human cancer; (3) studies on the role of viruses in the induction of human breast tumors, including continuation of studies on MPMV and related viruses; (4) studies on cocarcinogenesis including viral/viral, viral/chemical, and viral/hormonal; (5) the characterization of nucleic acids and their role in the induction of animal and human cancers, in particular, detection of transforming sequences in cellular nucleic acids and molecular genetic studies with DNA from human tumor cells; (6) studies on viral proteins as probes for viral gene expression in animals and humans; (7) studies on oncogenic viruses important to human ecology, for example, those derived from bovine, porcine, avian, etc. species; (8) continuation of collaborations on the breeding of endangered non-human primate species for tumor virus studies.

The delegates noted that the benefits of scientist exchange were substantially increased by better definitions of scientific interests and early availability of pertinent biographical materials of the participants before their laboratory visits. Both Sides recognized the importance of the continuing exchange of scientists for conducting joint research, for participating in conferences, seminars and other scientific sessions on the problem of cancer virology in accordance with the areas mentioned above. In particular, the members stressed the need for exchange of young scientists.

Future Meetings: American and Soviet tumor virologists will continue to participate in relevant conferences in their respective countries. It was proposed that the fifth meeting be held in Bethesda, Maryland, USA in October, 1977 in conjunction with the Annual Joint Working Conference of the Virus Cancer Program. The delegates also unanimously accepted a verbal proposal to hold small Working Group meetings to review areas of intense interest. They suggested that up to 3 meetings a year, attended by working scientists and perhaps 1-2 delegates, would agument the current exchange. Official permission to begin conducting such meetings was requested in a proposal dated July 9, 1976.

Summary of US-USSR scientist exchange program to date:

TO USSR:		Institutions Visited
Dr. Walter Nelson-Rees University of California, Berkeley	November, 1974	Inst. Expt'l & Clinical Oncology Inst. Medical Genetics Inst. Viral Preparations Inst. Virology Inst. Epidemiology (Gamaleya)
Dr. Robert Goldberg Viral Oncology Program, NCI	May, 1976	Inst. Expt'l Pathology & Therapy, Sukhumi (Dr. Lapin) and Institutes in Moscow and Leningrad
Dr. John Cicmanec Litton Bionetics, Inc. Kensington, Maryland	May, 1976	Inst. Expt'l Pathology & Therapy, Sukhumi (Dr. Lapin) and Institutes in Moscow and Leningrad
Dr. Sami Mayyasi Pfizer, Inc. Maywood, New Jersey	Proposed 1977	Inst. Expt'l Pathology & Therapy, Sukhumi (Dr. Lapin) and Institutes in Moscow and Leningrad
Dr. D.H. O'Rourke Dr. R.M. Baume University of Kansas, Lawrence	June, 1977	Inst. Expt'l Pathology & Therapy, Sukhumi (Dr. Lapin)
TO US:		
Dr. Viktor Zhdanov Ivanovsky Inst., Moscow	October, 1973	NCI scientists: VCP Annual Meeting
Dr. Frederick Seitz Petrov Research Inst., Leningrad	October, 1973	NCI scientists: VCP Annual Meeting
Dr. Konstantin Ilyin Gamaleya Inst., Moscow	February, 1974	NCI scientists: Flow Labs., Rockville: M.D. Anderson Hospital, Houston
Dr. T.A. Bektemirov Ivanovsky Inst., Moscow	October, 1974	NCI scientists: A. Einstein Coll. Med. (Dr. August): Columbia Univ. (Spiegelman)

TO US (continued):		Institutions Visited
Drs. E. Dzhikidze, Z. Shevtsova, V. Agrba, and Yakovleva Inst. Expt'l Pathology, Sukhumi	September, 1975	NCI scientists: VCP Annual Meeting: laboratories of Drs. Deinhardt, Spiegelman, Mayyasi: Sloan-Kettering Institute
Dr. N. Nosik Ivanovsky Inst., Moscow	September, 1975	Litton Bionetics (Dr. D. Fine): VCP Annual Meeting: Baylor Univ. (Dr. Melnick): Pfizer, Inc. (Dr. Mayyasi)
Dr. I. Kryukova Gamaleya Inst., Moscow	February, 1976	M.D. Anderson Hosp. (Dr. Bowen): Michigan Cancer Fdn. (Dr. Rich): Rockefeller Univ. (Dr. Hanafusa): NCI scientists
Prof. S.M. Klimenko Ivanovsky Inst., Moscow	September, 1976	NCI scientists: Inst. Cancer Research (Dr. Blumberg)
Dr. E. Bagley Kiev Inst. Expt'l and Clinical Oncology	March, 1977	NCI scientists: Fred Hutchinson Cancer Ctr. (Dr. Hakomori): Sloan-Kettering Inst. (Dr. Sonnenberg)
Dr. Z. Butenko Kiev Inst. Expt'l and Clinical Oncology	March, 1977	NCI scientists: laboratories of Dr. Spiegelman, W. Moloney, E. Cronkite, S. Mayyasi
Dr. S.A. Novokhatskiy Ivanovsky Inst., Moscow	May, 1977	NCI scientists: laboratory of Dr. R. Gallo: laboratories in Washington, D.C. area involved in large-scale production of human virus

This year under the Agreement, Prof. Sergei M. Klimenko, Head, Laboratory of Electron Microscopy, Ivanovsky Institute of Virology, Moscow, made a working visit to the US from September, 1976 to January, 1977. His problem was to identify regions of herpes simplex virus genome by heteroduplex mapping. This collaborative study was carried out with members of the Virus Tumor Biochemistry Section, Laboratory of DNA Tumor Viruses, Dr. George Vande Woude, Head. During his stay he had the opportunity to learn the methodology for obtaining and preparing nucleic acids for this study from Dr. J. Maizel and Dr. B. Peterlin. He also visited Dr. B.S. Blumberg at the Institute for Cancer Research in Philadelphia, PA and attended the VCP Annual Meeting in Hershey, PA.

Dr. E.A. Bagley, Senior Scientific Researcher, Institute of Oncology Problems, Kiev, Ukrainian SSR, made a working visit to the US from March, 1977 to June, 1977. Several weeks were spent in the laboratories of Dr. Peter Fischinger, Head, Virus Control Section, Laboratory of Viral Carcinogenesis, NCI, and Dr. Stuart Aaronson, Assistant Chief, Laboratory of RNA Tumor Viruses, NCI, to study methods for detecting viral genome expression in various cells. Later, he worked on a problem with Dr. S. Hakomori, Head, Division of Biochemical Oncology, Fred Hutchinson Cancer Research Center, Seattle, Washington, studying methods in glycoprotein and glycolipid identification. Before his return to the USSR in June, Dr. Bagley also visited the laboratory of Dr. Martin Sonnenberg, Sloan-Kettering Institute, New York City.

Another member of the staff of the Institute of Oncology Problems in Kiev, Dr. Z. Butenko, also began her visit in March, 1977. For about six weeks she worked in the laboratories of Dr. Robert Gallo, Chief, Laboratory of Tumor Cell Biology, Division of Cancer Treatment, NCI, and Dr. John Stephenson, Head, Viral Genetics Section, Laboratory of RNA Tumor Viruses, DCCP, NCI, studying mechanisms of viral carcinogenesis and immuno- and biochemical methods for the detection of tumor virus expression. Later, Dr. Butenko studied molecular methods for detecting cellular genome alterations in the laboratory of Dr. S. Spiegelman, Columbia University, New York City. Before leaving on May 6, she also visited briefly the Brookhaven National Laboratory (Dr. Eugene Cronkite), Upton, New York; Sloan-Kettering Institute (Dr. Malcolm Moore), New York City; Peter Bent Brigham Hospital (Dr. William Moloney), Boston; and Pfizer, Inc. (Dr. Sami Mayyasi), Maywood, New Jersey.

The Viral Oncology Program also assisted in arranging for appropriate host laboratories to receive several USSR scientists making working visits to the US under the aegis of the Fogarty International Center of the NIH: Dr. V.P. Yuferov, Ivanovsky Institute of Virology, Moscow, and Drs. T.I. Tikhonanko and V.N. Kalinin, also from the Ivanovsky Institute of Virology.

In May, 1976, Dr. Robert Goldberg (Viral Oncology Program, NCI) and Dr. John Cicmanec (Litton Bionetics, Inc., Kensington, MD) continued a collaboration initiated during an earlier visit to the US by several USSR scientists from the Institute of Experimental Pathology and Therapy (Sukhumi). During his stay, Dr. Goldberg introduced a new technique in molecular hybridization to the biochemistry laboratory at the Institute; performed DNA-RNA hybridizations using a ^{3}H-cDNA baboon type C virus probe prepared at the NCI and RNA from primate cell lines; purified cellular RNA by cesium chloride centrifugation, and type C viral RNA by oligo dT cellulose chromatography; tested purified baboon type C virus preparations for endogenous and exogenous reverse transcriptase activity; cloned tissue culture cell lines in agar and liquid media; and attempted to isolate a type C virus from M. arctoides by co-cultivation of two cell lines derived from this species of monkey with various mammalian cells. Dr. Cicmanec, visiting many different laboratories at the Center and speaking with most of the scientists, made an extensive analysis of the management of the baboon breeding colonies and experimental groups, and methods of feed preparation and analysis at the Institute.

Drs. Goldberg and Cicmanec also visited and presented seminars at the Ivanovsky Institute of Virology in Moscow; the Institute of Experimental and Clinical Oncology, Moscow; and the Petrov Institute, Leningrad.

Working visits are being planned for June, 1977 by Dennis H. O'Rourke and Robert M. Baume of the University of Kansas for a collaborative study with Professor Boris Lapin on "Risks of Lymphoma as a Possible Outgrowth of Inbreeding in Non-Human Primates." Dr. Michael H. Crawford, Director, Laboratory of Biological Anthropology, University of Kansas, and Dr. Lapin will direct this collaborative project. In general, the project will test the hypothesis that inbreeding reduces genetic variability which in turn increases susceptibility to viral infection. Blood specimens and morphological data from all of the available primates at the Sukhumi Primate Center will be collected. Dental casts, anthropometric measurements and dermatoglyphics will also be taken to measure the morphological effects of inbreeding. A more detailed description of this collaboration will appear in next year's annual report.

Dr. Sami Mayyasi, Assistant Director for Cancer Research at the J.L. Smith Memorial for Cancer Research, Pfizer, Inc., has submitted a proposal to visit the Soviet Union for a period of three weeks in September, 1977 under the US-USSR Agreement to lecture and discuss the large-scale production of oncogenic viruses. He plans to visit the primate center at Sukhumi as well as other laboratories in the USSR. A summary will appear in the next annual report.

US-France Cooperation. Since its inception in 1969, this Program has provided exchange of information and exchange of scientists between the two countries for the purpose of understanding the role of viruses in cancer. As noted in a previous report, new and expanded cooperation between the Institut Nationale de la Sante et de la Recherche Medicale (INSERM) and the National Cancer Institute (NCI) was initiated in December, 1975. At that time two special research efforts were launched; a third has been added, as follows:

(1) Viral Oncology
(2) Hormone Control and Cancer
(3) Clinical Trials for Cancer Treatment

The respective Program Coordinators for Viral Oncology, Dr. J.P. Levy (France) and Dr. J.B. Moloney (U.S.), will be responsible for the official exchanges of scientific information which will be implemented in the following ways: (a) convening a limited number of scientific seminars, conferences, workshops and annual review meetings of the respective program coordinators; (b) exchange of research information by providing reprints and preprints of current research projects; and (c) encouragement of personal exchange of information between French and American scientists. The exchange of resources will be encouraged between scientists through the existing funding mechanisms (grants, contracts or existing project funds). The Office of International Affairs, NCI and INSERM will provide a limited amount of funds in support of exchange of personnel for short-term (less than 3 months) and long-term (up to 12 months) periods.

The Viral Oncology Delegations within the USA/France Cancer Program held their first joint meeting in Bethesda, Maryland on September 15-16, 1976. Key members of the Viral Oncology Program staff presented the research thrust, resources, and fiscal information of the Virus Cancer Program (VCP). Copies of the most recent Progress Report and Catalogue of Resources of the VCP were given to the French delegates. The members briefly discussed two mechanisms of funding research projects in the U.S., the grant and the contract.

Delegates of both Sides then presented brief reports on the work being conducted in their own laboratories. The French scientists emphasized research that will be supported by their Cancer Program. Additionally, most delegates summarized the related studies in other laboratories within their respective countries.

The Coordinator for the French Delegation then reported on the activities in the other areas of interest in the USA/France exchange: hormones and cancer, and clinical trials. Finally, the Coordinator and the Chairman of the French Delegation reviewed, in detail, the mechanisms for dispersing funds for cancer research to French scientists. In particular, they described the activities of INSERM (Institut Nationale de la Sante et de la Recherche Medicale), and the CNRS (Centre Nationale de la Recherche Scientifique), giving examples of how a French scientist might obtain funds to support cancer-virus research under a contract (grant) in this system.

The U.S. Chairman offered support of the NCI to this collaborative effort. He described the organizational structure of the Viral Oncology Program with particular emphasis on the Virus Cancer Program including the funding of various cancer projects by area and commented on the procedures for applying for U.S. contracts. Examples of contract proposals were distributed to the French delegates.

A series of specific recommendations were set forth by both Sides for promoting scientist exchange, attendance and participation at scientific meetings, and information exchange. Finally, there was general agreement by both Sides that there was interest in collaborating in the following broad areas: RNA tumor viruses, DNA tumor viruses, and the role of viruses in certain human cancers such as leukemia, sarcoma and carcinoma. Some specific areas of collaboration suggested were bovine leukemia virus characterization; cocarcinogenesis studies; strain differences in herpesvirus isolates; characterization and definition of human isolates, as available; study of breast cancer isolates from animals and man; and studies on membrane changes.

The French Chairman suggested that he and other members of the Delegation would prepare a summary of ongoing research similar to the VCP Progress Report to provide an insight for other possible areas of collaboration between the USA and France.

It was agreed that the meetings of the Cancer Virology Delegations should be held annually, the next meeting to be held in France in late September, 1977. It was also recommended that delegation visits might be extended to several

days to allow visits to individual laboratories or to conduct a symposium in areas of mutual interest. The Chairmen stressed that all exchanges should be handled through their offices. Satisfaction was expressed by both Sides in the progress and understanding achieved at this first meeting.

At an informal meeting at the INSERM headquarters (April, 1977) the Chairmen of both Sides felt the cooperation was going very well and agreed that the next joint meeting would be held at the INSERM headquarters in Paris. The last week of September was an acceptable time; but the exact dates for the meeting were not defined. The Co-Chairmen agreed to conduct a one- or two-day symposium as a part of the meeting. The French Side proposed to invite other French virologists in addition to the delegates of both countries to participate in the scientific sessions. Both Sides agreed that during the administrative portion of the joint meeting recombinant-DNA studies and the use of small workshops would be discussed. The French Side was pleased to accept a list of scientist and resource exchanges presented by the U.S.

Ongoing and Proposed Working Visits under NCI/INSERM Agreement:

M. Hatanaka (NCI) to Institut Pasteur (P. Hosli) - April, 1977 (3 months)
N. Salzman (NIAID) to IRSC (R. Monier) - June, 1977 (2 months)
L. Warren (Wistar Inst.) to Inst. Pasteur (L. Montagnier)-July,1977 (6 months)
J. Yeh (Pfizer, Inc.) to Villejuif (C. Jasmin) - September, 1977 (3 months)
J.K. Youn (Villejuif) to Microbiological Associates (J. Rhim) - September,1977 (3 months)

US-Japan Cooperation: This cooperation was established in 1974 under an Agreement between the National Cancer Institute and the Japan Society for the Promotion of Science (JSPS). Several program areas identified for collaborative study have the following functions: to identify and recommend subject areas for joint activity and support scientific seminars under the program; to identify and recommend scientists and other professional personnel under the exchange of personnel program; to make recommendations regarding the inclusion of representatives from other countries in the seminars and other meetings sponsored under this program; to assess periodically the effectiveness of activities under this program; and to recommend necessary changes.

The Program Coordinators for Cancer Virology are Professors Robert McAllister (USA) and Yohei Ito (Japan). This year, in addition to scientist exchanges, a symposium on the "Origin and Function of Oncogenic Sequences in RNA Tumor Viruses" was held in Pasadena, California, May 31-June 1, 1977. Fifteen scientific papers were presented. In particular, the Japanese and American scientists reported on the characterization of sarcoma sequences in avian and mammalian cells. An account of the symposium will appear in next year's annual report.

Proposed Visit to U.S. under US-Japan Cancer Research Program:

A. Seto (Kyoto University) to NCI (Dr. R. Herberman) and Massachusetts General Hospital, Boston (P. Black and M. Hirsch) - September, 1977 (4 months).

MEETINGS

Eleventh Annual Joint Working Conference - Virus Cancer Program. The annual meeting of the Virus Cancer Program was held at Hershey, Pennsylvania on November 8-11, 1976. The following topics were discussed: endogenous viruses; mapping and structure of tumor viruses and their genes; genetic regulation of infection by type C viruses; biochemical and immunological evidence for viral involvement in cancers of primates, including man; transmissible viruses; functions of tumor viral proteins; prevention and control of viral neoplasia; and mechanisms of transformation. The official program of this meeting may be obtained from this Office upon request.

Guinea Pig L2C Leukemia: Immunological, Virological and Clinical Aspects. This symposium, held in Bethesda, Maryland on November 18-19, 1976, was organized and co-chaired by Drs. John S. Rhim and Ira Green, Microbiological Associates, Inc. Sixteen papers (to be published in Federation Proceedings) were presented and sixty participants discussed the unique advantages of guinea pig leukemia and endogenous guinea pig virus as a suitable model for studying leukemia. It was felt that further biological and biochemical studies on two different types of viruses found in leukemic guinea pig cells--retravirus-type RNA virus and herpes-type DNA virus--would be very important in understanding the role of these viruses in transformation and oncogenesis. Since the system is free from infectious viruses, studies can be done to determine the role of endogenous viruses in malignancy. The participants felt it would be rewarding to hold a follow-up meeting in about two years.

Third Conference on Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias. This conference, held on December 13-15, 1976 in Bethesda, and attended by over 120 scientists representing 7 countries, was devoted to pre-clinical and clinical studies of chemical and biological agents that enhance immune responses and increase host resistance to virus infection or tumor growth. Levamisole, Glucan, Thymosin, Pyran copolymer, Amphotericin B, and a new chemical agent BM 06002, were discussed in relation to their immunopotentiating capabilities. Evidence was presented that these immunomodulators exert their effects through the stimulation of macrophages and/or thymus-derived or bone marrow-derived lymphocytes. Levamisole probably acts by stimulating T-cells. When used with conventional chemical agents for the treatment of human lung carcinoma, malignant melanoma, breast carcinoma, Hodgkins disease, and immunodeficiency diseases, Levamisole stimulated immune T-cell responsiveness with beneficial effects.

Glucan was reported to stimulate bone marrow restoration as well as macrophage replication. Its use as an immunostimulator in the treatment of malignant melanoma causes regressions of dermal melanoma. Thymosin, similarly, was reported to be an effective T-cell stimulator and found to be beneficial when used in concert with chemotherapy in the treatment of both experimental and human tumors. Pyran copolymer, Amphotericin B and BM 06002 apparently increase host immune responsiveness to host tumor when used alone or in combination with chemotherapy or surgery. These agents are considered excellent candidates for clinical testing.

Workshop on Tumor Virus Antigens. On March 8-9, 1977, 45 scientists attended a Tumor Viral Immunology Workshop sponsored by the RNA Tumor Virus Studies Section, Collaborative Research Branch. During the informal sessions they resolved several problems in viral protein biochemistry: gene order, phosphorylation of proteins, precursor cleavage, and antigen location in virions. The participants identified differences in techniques and proposed certain experiments and exchanges of reagents. Because the field of protein characterization has reached a high degree of sophistication, they were optimistic that most problems could be solved in the near future.

On the second day, a committee composed of Drs. R. Arlinghaus, D. Bolognesi, R. Eisenman, E. Fleissner, R. Nowinski, S. Oroszlan, P. Roy-Burman and G. Vande Woude recommended a nomenclature system for oncornaviral proteins, as follows:

1. Precursor proteins: High molecular weight polyproteins known to cleave to lower molecular weight proteins associated with virions.

   Generic designation, followed by molecular weight (as determined in guanidine), followed by superscript gene origin if appropriate

   Generic designations: unmodified - Pr
   glycosylated percursor proteins - gPr
   phosphorylated precursor proteins - pPr

   Gene designations: gag - core proteins
   env - envelope proteins
   sarc or leuk - transforming proteins
   pol - polymerases

   e.g., $Pr80^{gag}$

2. Viral proteins: Proteins found in mature virions, which are not known to be further cleaved.

   Generic designation, followed by molecular weight (as determined in guanidine), followed by superscript gene origin, followed by location in virion in brackets, if appropriate.

   Generic designations: unmodified - p
   glycosylated - gp
   phosphorylated - pp (degree of phosphorylation noted by superscript o-n after first p)

   Gene designations: See under precursor proteins

   Virion locations: nucleoprotein - np (previously C)
   envelope - E

   e.g., $p12^{gag}$[E]

3. Polyproteins: High molecular weight proteins found in infected cells, not known to be cleaved further to proteins found in mature virions.

   Generic designation P, followed by molecular weight (as determined in guanidine), followed by superscript gene origin, followed by location in cell in brackets, if appropriate.

   Cell location designations: cell surface - cs
   cytoplasm - cy
   nucleus - nu
   virion - v

   e.g., $P86^{gag}$[cs]

It was recommended that the VOP provide the following reference reagents: purified oncornaviral proteins, monospecific antisera to these proteins, and certified, cloned virus preparations.

The host responses to viral proteins are only now beginning to be investigated by sophisticated techniques. Many areas were identified for future work, such as identification of cellular compartments responding to the various antigens in several species, and the protection, if any, associated with the various responses.

The participants felt it would be advantageous to hold a future workshop specifically on techniques available to define host responses, and another on developing vaccine protocols, identifying antigens to be used and immune responses to be monitored.

Workshop on Breast Cancer Virology. The first in a series of workshops to address specific problem areas in breast cancer virus research was held on March 28-29, 1977 at the N.I.H. The Mason-Pfizer monkey virus (MPMV) and related isolates were selected as the topic of Workshop I because of a number of reports in the literature suggesting that MPMV-related information was being detected in tumors of human breast cancer patients and because MPMV was the first oncornatype virus to be isolated from a primate breast carcinoma.

The workshop was divided into six sessions and included presentations on the morphological and biochemical characterization, molecular biology, immunology and virus production of MPMV, the langur monkey isolate, the squirrel monkey retravirus (SMRV) and a variety of MPMV-related isolates from human cell lines.

In the first sessions, a review of the initial isolation and characterization of MPMV, SMRV and the langur isolate was presented. Ultrastructural and biochemical properties of this distinct group of viruses, designated as type D, were compared. It was shown from hybridization and radioimmune assay studies that MPMV and the langur monkey virus have homology in RNA nucleotide sequences and host range, whereas the New World monkey isolate (SMRV) is endogenous and different in many ways from the other type D oncornaviruses. Although the precise origin of the Mason-Pfizer monkey virus is unknown, the virus apparently is not endogenous to rhesus monkeys since only a portion of the proviral

DNA sequences can be detected in tissues. Discussions on the MPMV-like particles isolated from some cultured human cell lines (i.e., the Graffi isolate, HEp-2, etc.) revealed that although some of the human isolates can be dismissed as HeLa cell-related, others can be distinguished from prototype MPMV. Several hypotheses were presented as to the possible origin of MPMV-related information in human cultured cells. It was agreed that further characterization of these isolates was necessary.

In later sessions dealing with the biology, biochemistry and immunology of MPMV and similar viruses, data was presented that infectious MPMV has been recently observed in milk obtained from adult rhesus monkeys inoculated intraductally as juveniles. Transmission of viral infection has been shown by foster nursing. It was reported that apparently MPMV can transform and persistently infect a variety of primate and human epithelial lines and a horse epidermis cell culture.

The final session dealt with the role of MPMV in human disease. The data on MPMV-RNA in human breast tumors, MPMV antibodies in the sera of breast cancer and leukemia patients and the presence of MPMV-p27 antigen in partially purified human breast extracts, is not sufficient to conclude that MPMV is involved in human breast cancer; further study was recommended.

International Symposium on Etiology and Control of Nasopharyngeal Carcinoma. On April 4-6, 1977, fifty scientists from 12 countries participated in a Symposium on Nasopharyngeal Carcinoma (NPC) held in Kyoto, Japan. Co-sponsored by the Viral Oncology Program, the World Health Organization-International Agency for Research on Cancer, and the Department of Microbiology, Kyoto University, the Symposium focused on the role of EBV in the etiology of NPC. Later this year, proceedings of this meeting will be published as a monograph by the IARC.

A summary of the presentations, grouped according to disciplines, follows:

Pathology: Since there is still considerable disagreement on the histological classification of NPC, the participants recommended continuing the classification. In doubtful cases, cytochemical analysis should be used to obtain a more definitive classification; additionally, slides of tissues from cases should be exchanged among several investigators. Tumors arising in individuals 19 years of age or younger should be distinguished from those occurring in the older age groups.

Genetics: It is important to determine the HLA type of cell lines obtained from NPC cases. The association between HLA type (A2S in 2) and serum antibodies to EBV in NPC patients needed additional study. The participants recommended measuring levels of C (Complement) in NPC patients and controls.

Clinical: The group proposed a new classification of the staging for NPC cases. This change does not affect the relationship of EBV serology.

Epidemiology: The participants suggested that childhood and adolescent NPC be compared to identify a possible relationship in the natural history of the disease and EBV infection and/or other cofactors. In particular, they recommended a 5-year prospective study of 5,000 Cantonese males of age 45-49 years. The group questioned whether reported differences in the time of occurrence of NPC in Chinese populations living in the U.S. and S.E. Asia may be attributed to changes in the traditional life style (socio-economic status) and/or environmental influences. Answers may be provided by analyzing cancer registries.

Immuno-Virology: The speakers recommended isolation and characterization of new virus isolates from NPC cases and emphasized the need for a comparative study of the disease as it occurs in various parts of the world. They felt it was important to search for viral receptors on permanent cell lines (NPC) of epithelial origin. Test for virus secretion should be limited to families in which cases of NPC have occurred.

Animal models for EBV studies are urgently needed. The limited availability of cotton-topped marmosets was a matter of great concern; steps to expand breeding programs should begin immediately. The group agreed that EBV studies should emphasize collaboration between pathologists and immuno-virologists and should include epitheliomas and all carcinomas of the nasopharynx and tonsils. A single source of purified, standardized antigens was recommended for studies on cellular immunology of NPC. *In vitro* and *in vivo* studies in this area should include EBV seropositive and sero-negative donor lymphocytes, and appropriate controls.

Workshop on Primates and Human Cancer. A Workshop on Primates and Human Cancer, held in San Antonio, Texas, April 11-13, 1977, arranged by the Southwest Foundation for Research and Education in collaboration with the Viral Oncology Program, provided Program scientists a tutorial session on the biology of non-human primates as applied to research on the etiology of human cancer. International experts in the ares of viral oncology, chemical carcinogenesis, radiation biology, anthropology, pathology, primatology and related areas presented recent advances in primate research. The participants stressed potential interaction of oncogenic viruses and chemical carcinogens as cofactors in the induction of malignancies. The sessions were recorded and will be printed by the Government Printing Office for distribution to members of the scientific community.

Third International Conference on Comparative Virology. The Third International Conference on Comparative Virology was held in Quebec, Canada, May 22-25, 1977. A summary of this meeting will appear in next year's annual report.

Third International Symposium on Oncogenesis and Herpesviruses. Held at Harvard University, Cambridge, Massachusetts, from July 25-30, 1977, this symposium was composed of the following sessions: DNA and herpesviruses, structure and association with cell-RNA; viral antigens and strain differences; cell-virus interactions--molecular event in permissive and semi-permissive systems; cell-virus interaction, cell transformation and properties of transformed cells; host-virus relationship--primary infections, latency, reactivation; host-virus relationship--development of lymphomas; host-virus relationship--leading to carcinoma development; and prospects for control of herpesvirus-related diseases. A summary and conclusions for this meeting will appear in next year's annual report.

VIIIth International Symposium on Comparative Research on Leukemia and Related Diseases. This Symposium was held in Amsterdam, The Netherlands, on August 22-26, 1977. The program included sessions on the epidemiology of leukemia and related diseases, genetic and immunologic factors in leukemogenesis, expression and detection of oncornaviruses in cells, herpesviruses and induction of leukemia and related diseases, nonviral leukemogenesis and cocarcinogenesis, cell proliferation and differentiation in relation to leukemogenesis, cell surface markers of leukemic cells, perspectives in diagnosis, prognosis and therapy in human leukemia, and new concepts in leukemia and related diseases. Proceedings of the Symposium will be issued in book form by Elsevier/North Holland Medical Press. A summary of this meeting will appear in next year's annual report.

7. PUBLICATIONS OF THE VIRAL ONCOLOGY AREA

1. Aaronson, S.A. and Schlom, J.: The search for RNA tumor viruses in human cancer. In Ariel, I. (Ed.): Progress in Clinical Cancer. New York, Grune & Stratton, Inc., 1976, p 51-63.

2. Aaronson, S.A. and Stephenson, J.R.: Viruses and the etiology of cancer. In Drew, W.L. (Ed.): Clinical Concepts in Virology. Philadelphia, F.A. Davis, p 251-292.

3. Aaronson, S.A. and Stephenson, J.R.: Intracellular and systemic regulation of biologically distinguishable endogenous type C RNA viruses of mouse cells. In Hanna, M.E. and Rapp, F. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, Vol 6, 1977, p 107-126.

4. Aaronson, S.A. and Stephenson, J.R.: Endogenous type C RNA viruses of mammalian cells. BBA Reviews on Cancer 458: 323-354, 1976.

5. Aaronson, S.A., Stephenson, J.R., Hino, S. and Cabradilla, C.: Endogenous type C RNA viruses of mouse cells: A model for the study of gene regulation in eukaryotes. In Criss, W.E., Ono, T. and Sabine, J.R. (Ed.): Proc VII Int Symp on Modified Cellular and Molecular Controls in Neoplasia, Dec 1975, Honolulu, Hawaii. New York, Raven Press, 1976, p 279-294.

6. Aaronson, S.A., Stephenson, J.R., Tronick, S.R. and Hino, S.: Immunological analysis of structural polypeptides of woolly monkey-gibbon ape type C viruses: Woolly monkey sarcoma virus. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 102-109.

7. Aaronson, S.A., Tronick, S.R. and Stephenson, J.R.: Type C RNA virus isolation from the black-tailed deer, Odocoileus hemionus: Evidence for endogenous viruses of New World origin. Cell 9: 489-494, 1976.

8. Ablashi, D.V., Aaslestad, H.G. and Easton, J.M.: Oncogenic herpesviruses. Proc of Int Workshop on Adv in Comparative Virology, Oct 1975, Munich, Germany. WHO Collaborating Center for Collection and Evaluation of Data on Comparative Virology (In Press).

9. Ablashi, D.V., Armstrong, G.R. and Easton, J.M.: Absence of horizontal transmission of Herpesvirus saimiri between experimentally infected and noninfected owl monkeys. Cancer Letters 2: 35-40, 1976.

10. Ablashi, D.V., Armstrong, G.R., Fellows, C., Easton, J.M. and Twardzik, D.R.: Evaluation of the effects of phosphonoacetic acid (PAA) and 2-deoxyd-glucose (2DG) on Herpesvirus saimiri (HVS) in vero cells. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

11. Ablashi, D.V., Easton, J.M. and Aaslestad, H.G.: Advances in research on oncogenic herpesviruses. Proc of Int Workshop on Adv in Comparative Virology, Oct 1975, Munich, Germany. WHO Collaborating Center for Collection and Evaluation of Data on Comparative Virology. Munich, UNI-Druck, 1976, p 291-326.

12. Ablashi, D.V., Easton, J.M. and Armstrong, G.R.: Oncogenic DNA viruses: Experimental animals and in vitro systems for propagation, assay, transformation and tumor induction. In Proc Int Symp Exp Animals and In Vitro Sys in Med Microbiol, Munich, 1976 (In Press).

13. Ablashi, D.V., Easton, J.M. and Glaser, R.: Animal models for nasopharyngeal carcinoma. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

14. Ablashi, D.V., Easton, J.M. and Guegan, J.H.: Herpesviruses and neoplasia in man and subhuman primates. Biomedicine 24: 286-305, 1976.

15. Ablashi, D.V., Easton, J.M., Levine, P.H., Krueger, G.R. and Connelly, R.: Immunological comparison of nasopharyngeal carcinoma and Burkitt's lymphoma from relatively high- and low-risk populations. Conf on Early Life in Man and Animals, Perugia Quadrennial Int Conf on Cancer, Perugia, Italy, Jun 1977 (In Press).

16. Ablashi, D.V., Oie, H.K., Armstrong, G.R., Didier-Fichet, M.L., Tronick, S.R., Heine, U. and Dalton, A.J.: Xenotropic properties of an isolate from murine Rauscher leukemia virus. J Med Primatol 5: 223-236, 1976.

17. Ablashi, D.V., Twardzik, D.R., Easton, J.M., Armstrong, G.R., Luetzeler, J., Jasmin, C. and Chermann, J.-C.: Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus-cell systems. Eur J Cancer (In Press).

18. Adams, A.: Epstein-Barr virus latency and bacteriophage P1 lysogeny-possible analogies. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 297-301, 1976.

19. Ahmed, M., Schidlovsky, G., Harewood, K.R., Manousos, M. and Mayyasi, S.A.: Expression of Mason-Pfizer and simian type C viruses in presence of 5-iododeoxyuridine and dexamethasone. J Natl Cancer Inst (In Press).

20. Ahmed, M., Yeh, J., Holden, H.E., Korol, W., Schidlovsky, G. and Mayyasi, S.A.: Characterization of Mason-Pfizer virus induced cell transformation in vitro. Archives of Virol (In Press).

21. Akira, D. and Takasugi, M.: Loss of specific natural cell-mediated cytotoxicity with absorption of natural antibodies from serum. Int J Cancer (In Press).

22. Albrecht, T., Nachtigal, M., St. Jeor, S.C. and Rapp, F.: Induction of cellular DNA synthesis and increased mitotic activity in Syrian hamster embryo cells abortively infected with human cytomegalovirus. J Gen Virol (In Press).

23. Al-Ghazzouli, I.K., Donahoe, R.M., Huang, K.-Y., Sarr, B., Peters, R.L. and Kelloff, G.J.: Immunity to virus-free syrgeneic tumor cell transplantation in the BALB/c mouse after immunization with homologous tumor cells infected with type C virus. J Immunol 117: 2239-2248, 1976.

24. Allen, P.T., Mullins, J.A., Saviolakis, G.A., Strickland, J.E., Fowler, A.K. and Hellman, A.: Direct isolation of xenotropic retraviruses from the uterus of the normal adult NIH Swiss mouse. Virology (In Press).

25. Andersson, M., Klein, G., Ziegler, J. and Henle, W.: Association of Epstein-Barr viral genomes with American Burkitt lymphoma. Nature 260: 357-359, 1976.

26. Andersson, M. and Lindahl, T.: Epstein-Barr virus DNA in human lymphoid cell lines: In vitro conversion. Virology 73: 96-105, 1976.

27. Andersson, M., Ziegler, J.L., Klein, G. and Henle, W.: Association of Epstein-Barr viral genomes with American Burkitt lymphoma. Lancet (In Press).

28. Andersson-Anvret, M., Forsby, N., Klein, G. and Henle, W.: The association between undifferentiated nasopharyngeal carcinoma and Epstein-Barr virus shown by correlated nucleic acid hybridization and histopathological studies. In: Etiology and Control of Nasopharyngeal Carcinoma. IARC Scientific Publications (In Press).

29. Aoki, T.: Serological approach to the detection of putative human type C RNA virus. In Gallo, R. (Ed.): Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology. Cleveland, Ohio, CRC Press, Inc. (In Press).

30. Aoki, T., Herberman, R.B., Hartley, J.W., Liu, M., Walling, M.J. and Nuun, M.: Surface antigens on transplantable tumor cell lines producing mouse type C viruses. J Natl Cancer Inst 58: 1069-1078, 1977.

31. Aoki, T., Liu, M., Walling, M.J., Bushar, G.S. and Brandchaft, P. B.: Specificity of naturally occurring antibodies in normal Gibbon serum. Science 191: 1180-1183, 1976.

32. Aoki, T., Liu, M., Walling, M.J., Bushar, G.S. and Hsu, K.C.: Human and gibbon natural antibodies to enevelope antigens of type C viruses from primates. Excerpta Medica (In Press).

33. Aoki, T., Walling, M.J., Bushar, G.S., Liu, M. and Hsu, K.C.: Natural antibodies in sera from healthy humans to antigens on surfaces of type C RNA viruses and cells from primates. Proc Natl Acad Sci USA 73: 2491-2495, 1976.

34. Armstrong, G.R., Orr, T., Ablashi, D.V., Pearson, G.I., Rabin, H., Loeb, W.F. and Valerio, M.G.: Characterization of Herpesvirus saimiri isolated from a chronically infected owl monkey. J Natl Cancer Inst (In Press).

35. Arnstein, P., Riggs, J.L., Oshiro, L.S., Huebner, R.J. and Lennette, E.H.: Induction of lymphoma and associated xenotropic type C virus in C57L mice by whole-body irradiation. J Natl Cancer Inst 1085-1090, 1976.

36. Arthur, L.O., Fine, D.L. and Bandyopadhyay, A.K.: Divalent cation specificities of in vitro produced mouse mammary tumor virus. J Virol (In Press).

37. Ash, J.F., Vogt, P.K., Singer, S.J.: The reversion from transformed to normal phenotype by inhibition of protein synthesis in normal rat kidney cells infected with a temperature-sensitive Rous sarcoma mutant. Proc Natl Acad Sci USA 73: 3603-3607, 1976.

38. Ashley, R.L., Cardiff, R.D. and Manning, J.S.: Characterization of a terminal deoxynucleotidyl transferase activity in mouse mammary tumor virus. Virology (In Press).

39. August, J.T. and Strand, M.: Type C oncornaviruses and autoimmunity. Arthritis Rheum (In Press).

40. Aurelian, L.: Sexually transmitted disease? The case for genital herpes. J Am Venereal Dis Assn (In Press).

41. Aurelian, L.: Coitus and Cancer. Proc Conf on Sexually Transmitted Diseases, New York Academy of Medicine, Nov 1974 (In Press).

42. Aurelian, L.: Coitus and Cancer. Bull NY Acad Med, Second Series, 52(8): 910-934, 1976.

43. Aurelian, L., Smith, M.F. and Cornish, J.D.: IgM antibody to a tumor-associated HSV-2 induced antigen (AG-4): Its use in locating the antigen in infected cells. J Natl Cancer Inst (In Press).

44. Aurelian, L., Strnad, B.C., Jacobs, R.P., Bell, R.B. and Smith, M.F.: Herpesvirus antigens and cell-mediated immunity in cervical cancer. In Crowell, R., Friedman, H. and Prier, J. (Ed.): Tumor Viruses and Immunity. Baltimore, Md., University Park Press (In Press).

45. Aurelian, L., Strnad, B.C. and Smith, M.F.: Immunodiagnostic potential of a virus-coded, tumor-associated antigen (AG-4) in cervical cancer. Cancer (In Press).

46. Austin, F., Takeichi, N., Robbins, M.L. and Boone, C.W.: Enhanced delayed hypersensitivity response to virus-augmented tumor specific antigens; Demonstration by radioisotopic footpad assay. 77th Annu Mtg Am Soc of Microbiology, New Orleans, La., May 1977.

47. Avila, J., Saral, R., Martin, R.G. and Khoury, G.: The temperature-sensitive defects in SV40 group D mutants. Virology 73: 89-95, 1976.

48. Axel, R. and Spiegelman, S.: Molecular virology of human neoplasia. In Sutnick, A.I. and Engstrom, P.E. (Ed.): Oncologic Medicine, Clinical Topics and Practical Management. University Press, 1976, p 1-22.

49. Baldwin, C.L., Sabel, F.L. and Henke, C.E.: Bedding disposal cabinet for containment of aerosols generated by animal cage cleaning procedures. Appl Microbiol 31 (In Press).

50. Baltimore, D.: The strategy of RNA viruses. In Zabriskie, J. (Ed.): The Harvey Lectures, series 70. New York, Academic Press, Inc., 1976 (In Press).

51. Baltimore, D., Silverstone, A.E., Kung, P.C., Harrison, T.A. and McCaffrey, R.P.: What cells contain terminal deoxynucleotidyl transferase? In Cunningham, A.J. (Ed.): The Generation of Antibody Diversity: A New Look. London, Academic Press, Inc., 1976 (In Press).

52. Baluda, M.A., Shoyab, M., Ali, M., Markham, P.D. and Drohan, W.N.: Restricted addition of proviral DNA in target tissues of chickens infected with avian myeloblastosis virus. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977, Vol 19: p 311-325.

53. Barahona, H., Daniel, M.D., Bekesi, J.G., Fraser, C.E.O., King, N.W., Hunt, R.D., Ingalls, J.K. and Jones, T.C.: In vitro suppression of Herpesvirus saimiri replication by phosphonoacetic acid. Proc Soc Exp Biol Med 154: 1977 (In Press).

54. Barahona, H., Daniel, M.D., Katz, S.L., Ingalls, J.K., Melendez, L.V. and King, N.W.: Isolation and in vitro characterization of a herpesvirus from a California ground squirrel (Citessus sp.). Lab Anim Sci (In Press).

55. Barahona, H., Melendez, L.V., Hunt, R.D. and Daniel, M.D.: The owl monkey (Aotus trivirgatus) as an animal model for viral diseases and oncogenic studies. Lab Anim Sci 26(6): 1104-1112, 1976.

56. Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Gag gene of mammalian type C RNA tumor viruses. Nature 262: 554-559, 1976.

57. Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Structural polypeptides of mammalian type C RNA viruses. J Biol Chem 251 (16): 4859-4866, 1976.

58. Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Evolutionary relationships between gag gene-coded proteins of murine and primate endogenous type C RNA viruses. Cell (In Press).

59. Barnes, R.D., Tuffrey, M., Holliday, J., Hilgers, J. and Souissi, T.: Murine leukaemia virus group-specific antigen in tumour-resistant tetraparental AKR CBA/H-T6 chimaeras. Br J Cancer 34: 23-34, 1976.

60. Baroudy, B.M., Papas, T.S. and Chirikjian, J.G.: Binding of tRNA trp beef to AMV DNA polymerase and its function as a heterologous primer. Proc Natl Acad Sci USA (In Press).

61. Beard, J.W. and Beard, D.: Oncogenicity of avian tumor viruses. In Beaudreau, G. (Ed.): The Biology of Tumor Viruses. Proceedings of the 34th Annual Biology Colloquium. Corvallis, Oregon, Oregon State University Press, 1975 (In Press).

62. Becker, L.E., Narayan, O. and Johnson, R.T.: Reduction in SV40 induced tumors by antisera to hamster fetal tissue. J Infect Dis (In Press).

63. Becker, S., Penyő, E.M. and Klein, E.: The natural killer cell in the mouse does not require H-2 homology and is not directed against group or type specific antigens of murine C-viral proteins. Eur J Immunol (In Press).

64. Becker, S. and Klein, E.: Decreased NK-effect in tumorbearing mice and its relation to the immunity against oncornavirus determined cell surface antigens. Eur J Immunol (In Press).

65. Becker, Y., Asher, Y., Cohen, Y., Weinberg-Zahlering, E. and Shlomai, J.: Phosphonoacetic acid resistant mutants of herpes simplex virus: Effect of phosphonoacetic acid on virus replication and in vitro DNA synthesis in isolated nuclei. Antimicrob Agents Chemother 11: 1977 (In Press).

66. Beemon, K., Faras, A.J., Haase, A.T., Duesberg, P. and Maisel, J.E.: Genomic complexities of murine leukemia and sarcoma reticulo-endotheliosis, and visna viruses. J Virol 17(2): 525-537, 1976.

67. Beemon, K.. and Keith, J.M.: Structure of Rous sarcoma virus RNA: 1) Localization of N6-methyladenosine; 2) the sequence of 23 nucleotides following the 5' capped terminus m7GpppGmp. In Baltimore, D., Huang, A.S. and Fox, C.E. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976, p 97-105.

68. Bekesi, J.G., Holland, J.F., et al: Immunotherapeutic efficacy of neuraminidase-treated allogeneic myeloblasts in patients with acute myelocytic leukemia. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 219-234.

69. Bekesi, J.G., Holland, J.F. and Roboz, J.P.: Specific immunotherapy with neuraminidase-modified leukemic cells. Med Clinics Symp on Advances in Treatment of Cancer, Sep 1977, Vol 61(5).

70. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Immunotherapy with neuraminidase-treated murine leukemia cells after cytoreductive therapy in leukemic mice. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

71. Bekesi, J.G., Roboz, J.P. and Holland, J.F.: Therapeutic effectiveness of neuraminidase-treated leukemia cells as immunogen in man and experimental animals with leukemia. Ann NY Acad Sci 277: 313, 1976.

72. Bekesi, J.G., Roboz, J.P., Thiem, T. and Holland, J.F.: Therapeutic effect of exogenous mouse interferon on murine leukemia and mammary tumor. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

73. Ben-Bassat, H., Goldblum, N., Mitrani, S., Goldblum, T., Yoffey, J.M., Cohen, M.M., Bentwich, Z., Ramot, B., Klein, E. and Klein, G.: Establishment in continuous culture of a new type of lymphocyte from a "Burkitt-like" malignant lymphoma (Line D.G.-75). Int J Cancer 19: 27-33, 1977.

74. Ben-Bassat, H., Goldblum, N., Mitrani, S., Klein, G. and Johansson, B.: Concanavalin A receptors on the surface membrane of lymphocytes from patients with African Burkitt's lymphoma and lymphoma cell lines. Int J Cancer 17: 448-454, 1976.

75. Bendas, C.M., Crowell, R.L. and Goldberg, R.J.: The spontaneous release of endogenous rat type C virus from myogenic cells in culture. In Vitro 1977 (In Press).

76. Bender, W. and Davidson, N.: Mapping of poly(A) sequences in the electron microscope reveals unusual structure of type C oncornavirus RNA molecules. Cell (In Press).

77. Benjamin, T.L.: Tumor viruses and cellular gene expression. Cancer Res 36: 4289-4290, 1976.

78. Bensinger, W.I., Robbins, K.C., Greenber, J.S. and Aaronson, S.A.: Different mechanisms for morphologic reversion of a clonal population of murine sarcoma virus-transformed nonproducer cells. Virology (In Press).

79. Benton, C.V., Johnson, R.W., Perry, A., Jones, W.I. and Shibley, G.P.: Tissue culture reageant quality assurance testing in a large-scale virus production laboratory. In Actin, R. and Lynn, D. (Ed.): Proc 1st Biannu Cell Culture Congress, Birmingham, Ala., Univ of Alabama Press, 1975 (In Press).

80. Bentvelzen, P. and Creemers, P.: Natural immunity to murine mammary tumor viruses. In Hanna, M. and Rapp, F.: Contemporary Topics in Immunobiology. New York, Plenum Press, 1977.

81. Benveniste, R.E., Callahan, R., Sherr, C.J., Chapman, V. and Todaro, G.J.: Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: Conservation of virogene sequences in related rodent species. J Virol 21: 849-962, 1977.

82. Ben-Zeev, A., Asher, Y. and Becker, Y.: Synthesis of herpes simplex virus-specified RNA by an RNA polymerase II in isolated nuclei in vitro. Virology (In Press).

83. Ben-Zeev, A. and Becker, Y.: Requirement of host cell RNA polymerase II in the replication of herpes simplex virus in alpha-amanitin-sensitive and -resistant cell lines. Virology 76: 246-253, 1977.

84. Berek, C., Taylor, B.A. and Eichmann, K.: Genetics of the idiotype of BALB/c myeloma S117: multiple chromosomal loci for VH genes encoding specificity for group A streptococcal carbohydrate. J Exp Med 144: 1164-1174, 1976.

85. Bergholz, C., Wolfe, L.G. and Deinhardt, F.: Oncogenicity of the C-type virus HL-23V in marmosets and characterization of virus isolated from an HL-23V-induced marmoset tumor: Comparison with simian sarcoma virus type 1. Primates in Medicine. Basel, S. Karger AG (In Press).

86. Bergholz, C., Wolfe, L.G., Deinhardt, F., Thakkar, B. and Marczynska, B.: Oncogenicity in marmosets of HL-23V, a type C oncornavirus isolated from human leukemic cells, and comparison with simian sarcoma virus, type 1 (SSV-1/SSAV-1). J Natl Cancer Inst (In Press).

87. Bernstein, I., Hellström, I., Hellström, K.E. and Wright, F.W.: Immunity to tumor antigens: Potential implications in human neuroblastoma. J Natl Cancer Inst 57: 711-715, 1976.

88. Bilello, J., Strand, M. and August, J.T.: Expression of viral envelope glycoprotein and transformation genes in cells transformed by a defective Kirsten murine sarcoma virus. Virology (In Press).

89. Bishop, J.M., Stehelin, D., Tal, J., Fujita, D., Roulland-Dussoix, D., Padgett, T., Varmus, H.E. and Spector, D.: The transforming gene of avian sarcoma virus. Symposium on the Molecular Biology of the Mammalian Genetic Apparatus, California Institute of Technology, Dec 1975 (In Press).

90. Bister, K., Varmus, H.E., Hunter, E. and Vogt, P.K.: Biological and biochemical studies on the inactivation of avian oncoviruses by ultra-violet irradiation. Virology 1976 (In Press).

91. Black, M.M.: Immunogenicity and MuMTV-like antigenicity of human breast cancer tissues. In Hanna, M.G., Jr. and Rapp, F. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Publishing Corp., 1977, Vol 6, p 239-262.

92. Black, M.M.: Structural, antigenic and biological characteristics of precancerous mastopathy. Cancer Res 36: 2596-2604, 1976.

93. Black, M.M.: Immunopathology of breast cancer. In Joachim, H.L. (Ed.): Pathobiology Annual 1977. New York, Appleton-Century Crofts (In Press).

94. Black, M.M. and Zachrau, R.E.: Immunotherapy of breast cancer. In Gallager, H.S., Leis, H.P., Jr., Snyderman, R.K. and Urban, J.A. (Ed.): The Breast. St. Louis, Mo., C.V. Mosby Company (In Press).

95. Black, M.M., Zachrau, R.E., Dion, A.S., Shore, B., Fine, D.L., Leis, H.P., Jr. and Williams, C.J.: Cellular hypersensitivity to gp55 of RIII-murine mammary tumor virus and gp55-like protein of human breast cancer. Cancer Res 36: 4137-4142, 1976.

96. Black, M.M., Zachrau, R.E. and Shore, B.: MuMTV-like characteristics of human breast carcinoma immunogens. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 559-564.

97. Black, P.H.: Lymphocyte virus interactions. Arthritis Rheum Suppl (In Press).

98. Black, P.H.: RNA viruses and human cancer. In Human Disease Caused by Viruses: Recent Developments. Oxford University Press (In Press).

99. Blair, D.G., Mason, W.S., Hunter, E. and Vogt, P.K.: Temperature-sensitive mutants of sarcoma viruses: Genetic recombination between multiple or coordinate mutants and avian leukosis viruses. Virology 75: 48-59, 1976.

100. Blank, K., Freedman, H. and Lilly, F.: T. lymphocyte response to Friend virus-induced tumor cell lines in mice of strains congenic at H-2. Nature (In Press).

101. Blank, K., Steeves, R. and Lilly, F.: The Fv-2r resistance gene in mice: Its effect on spleen colony formation by Friend virus-transformed cells. J Natl Cancer Inst 57: 925-929, 1976.

102. Bloomer, J.R., Bonkowsky, H.L., Ebert, P.S. and Mahoney, M.J.: Inheritance in protoporphyria. Comparison of haem synthetase activity in skin fibroblasts with clinical features. Lancet Jul 31: 226-228, 1976.

103. Bolognesi, D.P., Montelaro, R.C., Frank, H. and Schäfer, W.: A model for assembly of type C oncornaviruses. Science (In Press).

104. Bondurant, M., Hashimoto, S.I. and Green, M.: Methylation pattern of genomic RNA from Moloney murine leukemia virus. J Virol 19: 998-1005, 1976.

105. Bonnard, G.D., Manders, E.K., Campbell, D.A., Jr., Herberman, R.B. and Collins, M.J., Jr.: Immunosuppressive activity of a subline of the mouse EL-4 lymphoma I. Immunosuppressive virus causing the inhibitory activity. J Exp Med (In Press).

106. Boobis, A.R., Kouri, R.E., Nebert, D.W.: Genetic differences in the binding of benzo(a)pyrene metabolites to DNA in the mouse. In Nieburgs, H.E. (Ed.): 3rd Int Symp on Detection and Prevention of Cancer. New York, Marcel Dekker, Inc., Apr 1976 (In Press).

107. Boone, C.W.: Comment on comparing the membrane transport properties of "normal" & "transformed" BALB/3T3 cells. J Cell Physiol (In Press).

108. Boone, C.W. and Jacobs, J.B.: Sarcomas routinely produced from putatively non-tumorigenic BALB/3T3 and C3H/10 1/2 cells by subcutaneous inoculation attached to plastic platelets. J Supramol Struct (In Press).

109. Boone, C.W. and Takeichi, N.: Early detection of mouse connective tissue using implants of cells attached to small plastic plates. Proc of Am Cancer Assoc, Denver, 1977.

110. Boone, C.W. and Takeichi, N.: Spontaneous neoplastic transformation in vitro is a modified form of foreign body tumorigenesis. Cold Spring Harbor Symp, New York, 1977.

111. Boone, C.W., Takeichi, N., Paranjpe, M.S. and Gilden, R.V.: Vasoformative sarcomas arising from BALB/3T3 cells attached to solid substrates. Cancer Res (In Press).

112. Bowen, J.M., Dmochowski, L., Miller, M.F., Priori, E.S., Seman, G., Dodson, M.L. and Maruyama, K.: Implications of humoral antibody in mice and humans to breast tumor and mouse mammary tumor virus-associated antigens. Cancer Res (In Press).

113. Bowen, J.M., McBride, C.M., Miller, M.F. and Dmochowski, L.: The relationship of nucleolar antigens in malignant melanoma cells to disease prognosis. In Proc 9th International Pigment Cell Conference, Houston, Texas, 1975 (In Press).

114. Boyd, A.L.: Characterization of single-cell clonal lines derived from HSV-2 transformed mouse cells. Intervirology (In Press).

115. Boyd, A.L.: Cross-reacting HSV antigens in hamster and mouse cells transformed by UV-inactivated HSV-2. Infection & Immunity (In Press).

116. Boyse, E.A., Cantor, H. and Shen, F.W.: Nomenclature for antigens demonstrable on lymphocytes. Immunogenetics (In Press).

117. Brandchaft, P.E., Aoki, T. and Silverman, T.N.: Immunogenic and immunosuppressive components in soluble preparations from Moloney murine leukemia virus-induced tumor cells. Int J Cancer (In Press).

118. Brennan, P.J., Steiner, S., Courtney, R.J. and Skelly, J.: Metabolism of galactose in herpes simplex virus-infected cells. Virology (In Press).

119. Breznock, A.W., Harrold, J.B. and Kawakami, T.G.: Successful breeding of laboratory housed gibbons (Hylobates lar). Lab Anim Sci 27(2): 1977 (In Press).

120. Brown, J.M., Kong, Y.M., Peterson, W.D., Jr., Frost, P., Kaplan, J. and Lightbody, J.J.: Stimulation in primary and secondary MLC: Comparison between normal lymphocytes and T and B lymphoblast cell lines before and after glutaraldehyde treatment. Transplant Proc 8(3): 437-440, 1976.

121. Brown, J.P., Klitzman, J. and Hellström, K.E.: A microcytotoxicity for antibody binding to tumor cell surface antigens using 125 I-labelled protein A from Staphylococcus aureus. J Immunol Method 15: 57-66, 1977.

122. Brown, T.M., Jr., Heath, C.W., Jr., Lang, R.M., Lee, S.K. and Whalley, B.W.: Nasopharyngeal cancer in Bermuda. Cancer (In Press).

123. Bryant, M.L. and Klement, V.: Clonal heterogenity of wild mouse leukemia viruses: Host range and antigenicity. Virology 73: 532-536, 1976.

124. Bubbers, J.E. and Lilly, F.: Selective incorporation of H-2 antigenic determinants into Friend virus particles. Nature (In Press).

125. Buehring, G.C. and Williams, R.R.: Growth rate of human mammary epithelium in vitro varies with degree of dysplasia. Cancer Res 36: 3742-3747, 1976.

126. Buell, P., Henderson, B.E., Louie, E., Menck, H.R., Henle, W. and Pike, M.C.: Selected observations on the epidemiology of carcinoma of the nasopharynx and other pharyngeal cancers. Am J Epidemiol (In Press).

127. Burstein, S.R., Gerwin, B.I., Taylor, H. and Westley, J.: Formal catalytic mechanism of ascorbate oxidase. In Yasunobu, K.T., Mower, H.F. and Hayaishi, O. (Ed.): Iron and Copper Proteins. Proc of the First Symp on Iron and Copper Proteins. Advances in Exp Med Biol 74: 472-488, 1976.

128. Büttner, W., Veres-Molnar, S. and Green, M.: Preparative isolation and mapping of adenovirus 2 early messenger RNA species. J Mol Biol 107: 93-114, 1976.

129. Buynak, E.B., Weibel, R.E., McLean, A.A. and Hilleman, M.R.: Long-term persistence of antibody following Enders' original and more attenuated live measles virus vaccine (39564). Proc Soc Exp Biol Med 153: 441-443, 1976.

130. Byers, V.S., Levin, A.S., LeCam, L., Johnston, J.O. and Hackett, A.J.: Tumor specific transfer factor therapy in osteogenic sarcoma: A two year study. Ann NY Acad Sci 277: 621-627, 1976.

131. Cabradilla, C., Robbins, K.C. and Aaronson, S.A.: Mouse type C virus induction by translational inhibitors: Evidence for transcriptional derepression of a specific class of endogenous virus. Proc Natl Acad Sci USA 73: 4541-4545, 1976.

132. Cabral, G.A. and Schaffer, P.A.: Electron microscopic studies of temperature-sensitive mutants of herpes simplex virus type 2. J Virol (In Press).

133. Calafat, J., Hilgers, J., Van Blitterswijk, W.J., Verbeet, M. and Hagemar, Ph.C.: Antibody-induced modulation and shedding of mammary tumor virus antigens on the surface of GR ascites leukemia cells as compared with normal antigens. Presence of these antigens on the B and C particles. J Natl Cancer Inst (In Press).

134. Caldwell, G.G., Baumgartener, I., Carter, C., Cotter, S., Currier, R., Essex, M., Hardy, W.D., Jr., Olson, C. and Olsen, R.G.: Seroepidemiologic testing in man for evidence of antibodies to feline leukemia virus and bovine leukemia virus. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

135. Caldwell, G.G. and Heath, C.W., Jr.: Case clustering in cancer. Southern Medical Journal (In Press).

136. Callahan, R., Benveniste, R.E., Sherr, C.J., Schidlovsky, G. and Todaro, G.J.: A new class of genetically transmitted retravirus isolated from Mus cervicolor. Proc Natl Acad Sci USA 73: 3579-3583, 1976.

137. Callahan, R., Sherr, C.J. and Todaro, G.J.: A new class of murine retroviruses: Immunological and biological comparison of novel isolates from Mus cervicolor and Mus caroli. Virology 1977 (In Press).

138. Canaani, E., Duesberg, P. and Dina, D.: Clevage map of linear mouse sarcoma virus DNA. Proc Natl Acad Sci USA 74(1): 29-33, 1977.

139. Cardiff, R.D., Wellings, S.R. and Faulkin, L.J.: Biology of breast neoplasia. Cancer (In Press).

140. Cardiff, R.D., Young, L.J.T. and Ashley, R.L.: Hormone synergism in the in vitro production of the mouse mammary tumor virus. J Toxicology & Environmental Health 1: 117-129, 1976.

141. Carlson, J.V., Robey, W.G. and Ortwerth, B.J.: Lysine tRNA's associated with avian myeloblastosis virus 70S RNA. J Virol 19: 760-763, 1976.

142. Casagrande, J.C., Gerkins, V.R., Henderson, B.E., Mack, T.M. and Pike, M.C.: Exogenous estrogens and breast cancer in women with natural menopause. J Natl Cancer Inst (In Press).

143. Casey, J. and Davidson, N.: Rates of formation and thermal stabilities of RNA:DNA and DNA:DNA duplexes at high concentrations of formamide. Nucleic Acids Res, 1977 (In Press).

144. Cedar, H.: Annealing and hybridization properties of herpes simplex virus type 1 DNA. J Gen Virol 32: 337-347, 1976.

145. Cerny, J., Proffitt, M.R. and Black, P.H.: Immunological mechanisms in the pathogenesis of virus-induced murine leukemia. J Natl Cancer Inst (In Press).

146. Chan, E., Peters, W.P., Sweet, R.W., Ohno, T., Kufe, I.W., Spiegelman, S., Gallo, R.C. and Gallagher, R.E.: Characterization of a virus (HL23V) isolated from cultured acute myelogenous leukaemic cells. Nature 260: 266-268, 1976.

147. Chan, S.H., Chew, T.S. and Simons, M.J.: In vitro assay of lymphocyte-mediated immunity using 75 selenium-methionine: II. Lymphocyte activation by phytohaemagglutinin (PHA) in micro cultures. J Immunol Methods (In Press).

148. Chan, S.H., Chew, T.S., Yap, J. and Simons, M.J.: In vitro assay of lymphocyte-mediated immunity using 75 selenium-methionine: I. Lymphocyte activation by phytohaemagglutinin (PHA) in macrocultures. Cell Tissue Kinetics (In Press).

149. Chan, S.H., Hong-Goh, E., Chew, T.S., Levine, P.H. and Simons, M.J.: Immunological status of NPC patients in Singapore. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

150. Chan, S.H., Simons, M.J., Chew, T.S., De Thé, G. and Shanmugaratnam, K.: Immunological aspects of nasopharyngeal carcinoma (NPC) - In vitro lymphocyte hyporesponsiveness to stimulation with phytohaemagglutinin (PHA). Int J Cancer (In Press).

151. Chan, S.H., Wallen, W.C., Levine, P.H., Periman, P. and Perline, E.: Lymphocyte response to EBV-associated antigens in infectious mononucleosis, Hodgkin's and non-Hodgkin's lymphoma patients using the leukocyte adherence inhibition assay. Int J Cancer 19(3): 356-363, 1977.

152. Chan, S.H., Wallen, W.C., Levine, P.H. and Soares, P.: Detection of immunity to possible viral and tumor-associated antigens in breast cancer and melanoma using the leukocyte adherence inhibition assay. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

153. Chander, S., Devare, S.G. and Stephenson, J.R.: Comparison of radioimmunoassay and Bendixen's hematological evaluation of adult and calf forms of bovine lymphosarcoma. Proc of Int Conf on Bovine Leukemia, Brussels, Nov 1976 (In Press).

154. Charman, H.P., Gardner, M.B., McAllister, R.M., Kim, N. and Gilden, R.V.: Humoral immune responses of cats to mammalian type C virus p30. Int J Cancer (In Press).

155. Charman, H.P., Kim, N., Gilden, R.V., Hardy, W.D., Jr. and Essex, M.: Humoral immune responses of cats to feline leukemia virus: Comparison of responses to the major structural protein, p30, and to a virus specific cell membrane antigen (FOCMA). J Natl Cancer Inst (In Press).

156. Chatigny, M.A. and West, D.L.: Ventilation rates: Theoretical and practical considerations. In Iodine, L.S. (Ed.): Laboratory ventilation for hazard control, Cancer Research Safety Monograph. U.S. Department of Health, Education and Welfare, National Institutes of Health No. 77-1293, 1977 (In Press).

157. Cheeseman, S.H., Rinaldo, C.R., Jr. and Hirsch, M.S.: Antiviral action of interferon in man: Use of interferon in cytomegalovirus infections in man. Tex Rep Biol Med (In Press).

158. Chen, H.W., Heiniger, H.J. and Kandutsch, A.A.: Stimulation of sterol and DNA synthesis in leukemic blood cells by low concentration of phytohemagglutinin. Exp Cell Res (In Press).

159. Chen, H.W., Kandutsch, A.A. and Heiniger, H.J.: The role of cholesterol in malignancy. In Homburger, F. (Ed.): Progress in Cancer Research. Basel, S. Karger AG (In Press)

160. Chen, Y.C., Hayman, M.J. and Vogt, P.K.: Properties of mammalian cells transformed by temperature-sensitive mutants of avian sarcoma virus. Cell 1976 (In Press).

161. Chinnadurai, G., Jeng, Y.-H., Gilead, Z. and Green, M.: Identification of early proteins induced by highly oncogenic human adenovirus 12 during lytic infection and in hamster tumors. Biochem Biophys Res Commun 74(3): 1199-1205, 1977.

162. Chinnadurai, G., Rho, H.M., Horton, R.B. and Green, M.: mRNA from the transforming segment of the adenovirus 2 genome in productively infected and transformed cells. J Virol 20: 255-263, 1976.

163. Chirigos, M.A.: In vitro and in vivo studies with thymosin. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

164. Chirigos, M.A., Mohr, S.J. and Chaparas, S.D.: Combined modality treatment with levamisole against neoplasias and protective effects. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

165. Chirigos, M.A. and Theodore, T.: The effect of immune modulators on the oncogenic process: Adjuvant effect of staphylococcus aureus in treating murine leukemia. In Friedman, H. and Southam, C. (Ed.): International Conference on Immunobiology of Cancer. New York, New York Academy of Sciences, 1975 (In Press).

166. Chirikjian, J.G., Samuel, K., Papas, T.S.: Release of the alpha subunit from AMV DNA polymerase. Proc X Int Congress of Biochemistry (In Press).

167. Cho, H.Y., Cutchins, E.C., Rhim, J.S. and Huebner, R.J.: Revertants of human cells transformed by murine sarcoma virus. Science 194: 951-953, 1976.

168. Chopra, H.C.: An oncorna-type virus from monkey breast tumor. In Melnick, J. (Ed.): Progress Medical Virology. Basel, S. Karger AG, 1976, Vol 22 (In Press).

169. Clements, G., Klein, G., Zeuthen, J. and Povey, S.: The selection of somatic cell hybrids between human lymphoma cell lines. Somatic Cell Genetics 2(4): 309-324, 1976.

170. Clewley, J.P., Bishop, D.H.L., Kang, C.-Y., Coffin, J., Schnitzlein, W.M., Reichmann, M.E. and Shope, R.E.: Oligonuclectide fingerprints of RNA species obtained from rhabdoviruses belonging to the vesicular stomatitis virus subgroup. J Virol 1977 (In Press).

171. Cloyd, M.W., Bolognesi, D.P. and Bigner, D.D.: Immunofluorescent analysis of expression of the RNA tumor virus major glycoprotein, gp71, on surfaces of virus-producing murine and other mammalian species cell lines. Cancer Res 37: 922-930, 1977.

172. Cloyd, M.W., Bolognesi, D.P. and Bigner, D.D.: Immunofluorescent analysis of expression of the RNA tumor virus major glycoprotein, gp71, on the surfaces of normal murine cells. Cancer Res 37: 931-938, 1977.

173. Cochrane, C.G. and Dixon, F.J.: Immune complex injury. In Samter, M. (Ed.): Immunological Diseases. New York, Little, Brown and Co. (In Press).

174. Cockerell, G.L., Hoover, E.A., Krakowka, S.G., Olsen, R.G. and Yohn, D.S.: Lymphocyte mitogen reactivity and enumeration of circulating bond T cells during feline leukemia virus infection in the cat. J Natl Cancer Inst 57: 1095-1099, 1977.

175. Cockerell, G.L., Krakowka, S.G., Hoover, E.A., Olsen, R.G. and Yohn, D.S.: Lymphocyte mitogen reactivity in feline leukemia virus infected cats and identification of feline T and B lymphocytes. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 81-83 .

176. Cockerell, G.L., Krakowka, S.G., Hoover, E.A., Olsen, R.G. and Yohn, D.S.: Characterization of feline T and B lymphocytes and identification of an experimental induced T cell neopolasm in the cat. J Natl Cancer Inst 57: 907-913, 1976.

177. Coffin, J. and Haseltine, W.: Terminal redundancy and origin of replication of Rous sarcoma virus. Proc Natl Acad Sci USA 74: 1977.

178. Colcher, D., Drohan, W. and Schlom, J.: Mason-Pfizer virus RNA genome: Relationship to the RNA of morphologically similar isolates and other oncornaviruses. J Virol (In Press).

179. Collins, J.J., Bolio, L.D., Denny, T.P. and Bolognesi, D.P.: Interspecies determinants of Friend leukemia virus antigens involved in cytolysis of virus-producing cells. J Virol 21: 113-118, 1977.

180. Connan, G., Suskind, R.G. and Rabotti, G.F.: Effect of low doses of actinomycin D on RNA synthesis in chick embryo fibroblasts transformed by Rous sarcoma virus. Biochimie 59: 105-109, 1977.

181. Cordell, B., Stavnezer, E., Friedrich, R., Bishop, J.M. and Goodmar, H.M.: Nucleotide sequence that binds primer for DNA synthesis to the avian sarcoma virus gerome. J Virol 19(2): 548-558, 1976.

182. Cosgrove, M., Benton, B. and Henderson, B.E.: Male genitourinary abnormalities and maternal diethylstibestrol. J of Urol (In Press).

183. Courtney, R.J.: Immunological and biochemical characterization of individual polypeptides induced by herpes simplex virus types 1 and 2. In Borek, C. and Kirg, D.W. (Ed.): Herpes Viruses (Advances in Pathobiology). New York, Stratton Intercontinental Medical Book Corporation (In Press).

184. Courtney, R.J., Schaffer, P.A. and Powell, K.L.: Synthesis of virus-specific polypeptides by temperature-sensitive mutants of herpes simplex virus type 1. Virology 75: 306-318, 1976.

185. Creemers, P.: The role of leukocyte subpopulations in the indirect leukocyte adherence inhibition assay in the mammary tumour virus system. Eur J Immunol 7: 48, 1977.

186. Crittenden, L.B.: The epidemiology of avian lymphoid leukosis. Cancer Res (In Press).

187. Crittenden, L.B., Motta, J.V. and Smith, E.J.: Genetic control of RAV-0 production in chickens. Virology 76: 90-97, 1977.

188. Crittenden, L.E. and Robinson, H.L.: Genetic control of endogenous avian tumor virus expression. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

189. Cuatico, W., Cho, J.-R. and Spiegelman, S.: Molecular evidence for a viral etiology of human CNS tumors. Acta Neurochir 35: 149-160, 1976.

190. Cuttner, J., Holland, J.F., Eekesi, J.G. and Silver, R.T.: Immunotherapy of acute myelocytic leukemia with MER. N Engl J Med (In Press).

191. Dalianis, T., Andersson, B. and Klein, G.: Separation of cells with different histocompatibility (H-2) antigens by passage through cellular immunoadsorbent columns. Eur J Immunol (In Press).

192. Dalianis, T., Klein, G. and Andersson, B.: Column separation of viral capsid antigen (VCA) positive from VCA negative cells in an Epstein-Barr virus (EBV) producing lymphoid line. Int J Cancer 19: 460-467, 1977.

193. Daniel, M.D., Barahona, H., Melendez, L.V., Hunt, R.D. and Forbes, M.: Prevention of fatal herpes infections in owl and marmoset monkeys by vaccination. Proc of the 6th Int Cong of Primatology, Aug 1976, Cambridge, England (In Press).

194. Daniel, M.D., Silva, D., Jackman, D., Sehgal, P., Baggs, R., Hunt, R.D. and Melendez, L.V.: Reactivation of squirrel monkey heart isolate (Herpesvirus saimiri strain) from latently infected human cell cultures and induction of malignant lymphoma in marmoset monkeys. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

195. Das, M.R., Padhy, L.C., Kosky, R., Sirsat, S. and Rich, M.A.: Human milk samples from different ethnic groups contain RNAse that inhibits, and plasma membrane that stimulates reverse transcription. Nature 262: 802-805, 1976.

196. Davis, J., Gilden, R.V. and Croszlan, S.: An antigenically related trypticpolypeptide from several mammalian type C RNA virus p30s. Intervirology, p 392-395.

197. Davis, J., Scherer, M.A., Tsai, W.P. and Long, C.: A low molecular weight Rauscher leukemia virus protein with preferential binding for single-stranded RNA and DNA. J Virol (In Press).

198. Davoli, D., Ganem, D., Nussbaum, A.L., Fareed, G.C., Howley, P., Khoury, G. and Martin, M.A.: Genome structures of reiteration mutants of simian virus 40. Virology 77: 110-124, 1977.

199. Day, N.E. and Simons, M.J.: Disease susceptibility genes - their identification by multiple case family studies. Tissue Antigens 8: 109-119, 1976.

200. Dean, J.H., McCoy, J.L., Cannon, G.B., Leonard, C.M., Perlin, A., Kreutner, A., Oldham, R.K. and Herberman, R.B.: Cell-mediated responses of breast cancer patients to autologous tumor-associated antigens. J Natl Cancer Inst 58(3): 549-556, 1977.

201. Dean, J.H., McCoy, J.L., Cannon, G.B., Weese, J.L., Oldham, R.K. and Herberman, R.B.: Lymphocyte proliferative responses of patients with carcinoma of breast or lung to mitogens, allo-antigens and tumor-associated antigens. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

202. Deinhardt, F.: Eine immunologische tumorprophylaxe oder therapie denkbar. Immunologie & Gesselschaft (In Press).

203. Deinhardt, F., Falk, L.A., Wolfe, L.G., Schudel, A., Nonoyama, M., Lai, P., Lapin, B.A. and Yakoleva, L.: Susceptibility of marmosets to Epstein-Barr virus-like babcon herpesviruses. Primates in Medicine. Basel, S. Karger AG (In Press).

204. Deinhardt, F., Wolfe, L.G. and Ogden, J.D.: The importance of rearing marmoset monkeys in captivity for conservation of the species and for biomedical research. Proc of the First Inter-American Conf on Conservation and Utilization of American Nonhuman Primates in Biomedical Research 1975. Washington, D.C., Pan-American Health Organization/WHO, Sci Publ No. 317, 1976, p 65-71.

205. DeLarco, J. and Todaro, G.J.: Membrane receptors for mouse leukemia viruses: Characterization using the purified viral envelope glycoprotein, gp71. Cell 8: 365-371, 1976.

206. Del Villano, B.C., Croker, B.P., McConahey, P.J. and Dixon, F.J.: Immunopathogenicity and oncogenicity of murine leukemia viruses. II. Infection of mice and rats with Scripps leukemia virus. Am J Pathol (In Press).

207. Del Villano, B.C., Kennel, S.J. and Lerner, R.A.: Biological and structural pleomorphism of the oncornavirus envelope glycoprotein, gp70. In Hanna, M.G. and Rapp, F. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, Vol 6 (In Press).

208. Del Villano, B.C. and Lerner, R.A.: Relationship between the oncornavirus gene product gp70 and a major protein secretion of the mouse genital tract. Nature (In Press).

209. Del Villano, B.C., Lerner, R.A., Kennel, S.J. and Elder, J.H.: Expression of an oncornavirus gene product in the mouse genital tract. Proc 3rd Annu NICHD Workshop on the Testis. New York, Raven Press (In Press).

210. Del Villano, B.C., Nave, B. and Lerner, R.A.: Oncornavirus genes and host proteins. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

211. De Noronha, F., Schäfer, W., Baggs, R. and Bolognesi, D.P.: Prevention of oncornavirus-induced sarcomas in cats by treatment with anti-viral antibodies. Nature (In Press).

212. Desgranges, C. and de Thé, G.: Presence of Epstein-Barr virus (EBV) specific IgA in saliva of nasopharyngeal carcinoma (NPC) patients: Their activity, origin and possible clinical value. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

213. Desgranges, C., de The, G., Ho, J.H.C. and Ellouz, R.: Neutralizing EBV specific IgA in throat-washings of nasopharyngeal carcinoma (NPC) patients. Int J Cancer (In Press).

214. Desgranges, C., Lenoir, G., de The, G., Seigneurin, J.M., Bilgers, J. and Dubouch, P.: In vitro transforming activity of EBV - I. Establishment and properties of two EBV strains (M81 and M72) produced by immortalized Callithrix jacchus lymphocytes. Biomedicine 25: 349-352, 1976.

215. De Thé, G.: Is Burkitt's lymphoma (BL) related to a perinatal infection by Epstein-Barr virus (EBV)? Lancet 335-338, 1977.

216. De Thé, G.: Viruses as causes of some human tumors? Results and prospectives of the epidemiological approach. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York (In Press).

217. De Thé, G.: Epidemiologie et nature de l'association entre virus herpes (EBV et herpes simplex) et certaines tumeurs humaines. Bull Cancer 63: 417-426, 1976.

218. De Thé, G.: The Epstein-Barr virus (EBV), its transforming activity in vitro, its oncogenic potential in vivo and its likelihood of being the first proven human cancer virus: Proc Symp on Viruses and Exchange of Genetic Information in Prokaryotes, Moscow, May 1975 (In Press).

219. De Thé, G.: Epstein-Barr virus behavior in different populations and implications for control of Epstein-Barr virus-associated tumors. Cancer Res 36: 692-695, 1976.

220. De Thé, G., Brugère, J., Cammoun, N., Ellouz, R., Henderson, B., Ho, J.H.C., Klein, G., Lavoué, M.F., Muenz, L., Schwaab, G., Shanmugaratnam, D. and Simons, M.J.: Comparative EBV serological data and markers in nasopharyngeal carcinomas from high, intermediate and low risk areas. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

221. De Thé, G., Day, N.E., Geser, A., Ho, J.H.C., Simons, M.J., Sohier, R., Tukei, P. and Vonka, V.: Epidemiology of the Epstein-Barr virus infection and associated tumours in man, a tool for aetiology and control. In Clemmesen, J. and Yohn, I.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press)

222. De Thé, G. and Geser, A.: Etudes epidemiologiques prospectives et nature de l'association entre le virus d'Epstein-Barr (EBV) et le lymphôme de Burkitt (BL). C R Acad Sci (d) (Paris) (In Press).

223. De The', G., Geser, A. and Munoz, N.: Viruses and human neoplasia. In Symington, T. and Carter, R.I. (Ed.): Scientific Foundations of Oncology. London, Wm. Heinemann Medical Books Ltd. (In Press).

224. De The', G., Ho, J.H.C. and Muir, C.: Nasopharyngeal carcinoma. In Evans, A.S. (Ed.): Viral Infections in Man: Epidemiology and Control. New York, Plenum Press (In Press).

225. De The', G. and Lenoir, G.: Comparative diagnosis of Epstein-Barr virus related diseases: Infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma. In Kurstak, E. (Ed.): Comparative Diagnosis of Viral Diseases. New York, Academic Press, 1977 (In Press).

226. De The', G., Vuillaume, M., Giovanella, B.C. and Klein, G.: Epithelial characteristics of tumor cells in nasopharyngeal carcinoma passaged in nude mice: Ultrastructure. J Natl Cancer Inst 57: 1101-1105, 1976.

227. Devare, S.G., Chander, S., Saragh, F.S. and Stephenson, J.R.: Evaluation of radioimmunoprecipitation for detection of bovine leukemia virus infection in domestic cattle. J Immunol (In Press).

228. Devare, S.G., Stephenson, J.R., Sarma, P.S. and Aaronson, S.A.: Bovine lymphosarcoma: Development of a sensitive radioimmunologic technique for detection of the etiologic agent. Science 194: 1428-1430, 1976.

229. Dina, D., Beemon, K. and Duesberg, P.: The 30S Moloney sarcoma virus RNA contains leukemia virus nucleotide sequences. Cell 9(2): 299-309, 1976.

230. Dion, A.S., Heine, U., Pomenti, A.A., Korb, J. and Weber, G.H.: Electrophoretic analysis of the molecular weight of murine mammary tumor virus RNA. J Virol (In Press).

231. Dion, A.S. and Moore, D.H.: Some biochemical aspects of murine mammary tumor virus and the putative human mammary tumor virus. In Gallo, R.C. (Ed.): The Molecular Biology of Neoplasia: Focus of Current Cancer Research. Cleveland, Ohio, CRC Press, Inc. (In Press).

232. Dix, R.D. and Courtney, R.J.: Effects of cytochalasin B on herpes simplex virus type 1 replication. Virology (In Press).

233. Dixon, F.J., Croker, B.P., Del Villano, B.C., Jensen, F.C. and Lerner, R.A.: The possible role of oncornaviruses in murine lupus. In Dumonde, D.C. (Ed.): Infection and Immunology in the Rheumatic Diseases. London, Blackwell Scientific Publications, 1976, p 275-279.

234. Dixon, F.J., Jensen, F.C., McConahey, P.J. and Croker, B.P.: Oncornaviruses and immunologic disease. In Miescher, P.A. (Ed.): Proc VII Symp of Immunopathology. Germany (In Press).

235. Dmochowski, L.: Viral type A and type B hepatitis--morphology, biology, and immunology--a review. Am J Clin Pathol (In Press).

236. Dmochowski, L.: Morphological, biological, immunological and biochemical studies on bone tumors of animals and man. In Grundmann, E.: Recent Results in Cancer Research, Vol 54. Berlin, Springer-Verlag, 1976, p 166-184.

237. Dmochowski, L. and Bowen, J.M.: Transforming factors in human sarcoma cells in tissue culture. Journal of Clinical Orthopaedics and Related Research (In Press).

238. Dmochowski, L., Bowen, J.M., Myers, E., Priori, E.S., Miller, M.F., Seman, G., Chan, J.C., Dodson, M.L., Scanlon, M.D., Ohtsuki, Y. and Yoshida, H.: Comparative studies of type C, type B, and M-PM oncornaviruses. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

239. Dmochowski, L. and Horoszewicz, J.S.: Viral oncology of prostatic cancer. Seminars in Oncology 3(2): 141-150, 1976.

240. Dölken, G. and Klein, G.: A solid - phase radioimmunoassay for Epstein-Barr virus (EBV)-associated membrane antigen prepared from B95-8 cell culture supernatants. J Natl Cancer Inst (In Press).

241. Dölken, G. and Klein, G.: Expression of Epstein-Barr virus-associated membrane antigen in Raji cells superinfected with two different virus strains. Virology 70: 210-213, 1976.

242. Doller, E.W.: Evidence for an inhibitor of herpes simplex virus in transformed hamster cells. Proc Soc Exp Biol Med 154: 168-170, 1977.

243. Doller, E.W. and Rapp, F.: Effect of levamisole on metases by herpesvirus-transformed cells. In Chirigos, M.A. (Ed.): Control of Neoplasia by Modulation of the Immune System, Progress in Cancer Research and Therapy. New York, Raven Press, 1977, Vol 2, p 97-105.

244. Dorizzi, M., Ortiz-Muniz, G., Lopez, D.M., Sigel, M.M. and Epstein, R.S.: Increase in the proportion of cells with the C'3 receptor in BALB/c mice bearing mammary tumors. Int J Cancer 16: 1015-1021, 1975.

245. Dosik, H., Wachtel, S.S., Kahn, F., Spergel, G. and Koo, G.C.: Evidence for the presence of Y-chromosomal genes in a pherotypic male with a 46 XX karyotype. J Am Med Assoc 236(22): 2505-2508, 1976.

246. Drew, S.I., Terasaki, P.I., Billing, R.J., Bergh, O.J., Minowada, J. and Klein, E.: Group-specific human granulocyte antigens on a chronic myelogenous leukemia cell line with a Philadelphia chromosome marker. Blood (In Press).

247. Drohan, W., Colcher, D., Schochetman, G. and Schlom, J.: Distribution of Mason-Pfizer virus-specific sequences in the DNA of primates. J Virol (In Press).

248. Drohan, W., Kettmann, R., Colcher, D. and Schlom, J.: Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in the C3H and RIII mouse strains. J Virol 21: 986-995, 1977.

249. Dube, S., Kung, H.J., Bender, W., Davidson, N. and Ostertag, W.: Size, subunit composition and secondary structure of the Friend virus genome. J Virol 20(1): 264-272, 1976.

250. Duesberg, P., Wang, L.-H., Beemon, K., Kawai, S. and Hanafusa, H.: Sequences and functions of Rous sarcoma virus RNA. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977, p 327-340.

251. Duesberg, P., Wang, L.-H., Mellon, P., Mason, W.S. and Vogt, P.K.: Towards a complete genetic map of Rous sarcoma virus. Anim Virology IV: 107-125, 1976.

252. Dumont, J., Liabeuf, A., Menle, W., Feingold, N. and Kourilsky, F.M.: Anti-EBV antibody titers in non-Hodgkin lymphomas. Int J Cancer 18: 14-23, 1976.

253. Dupont, E., Opelz, G., Gustafsson, L.A., Mikulski, S.M. and Terasaki, P.I.: Depressed effector cell function in antibody-dependent cell-mediated cytotoxicity in kidney allograft recipients. Transplantation 23: 165-168, 1977.

254. Duran-Troise, G., Bassin, R.H., Rein, A. and Gerwin, B.I.: Loss of Fv-1 restriction in BALB/3T3 cells following infection with a single N tropic murine leukemia virus particle. Cell 10(3): 479-488, 1977.

255. East, J.L., Knesek, J.E., Chan, J.C., Maruyama, K., Priori, E.S. and Dmochowski, L.: Sequence relatedness of mammalian viral RNA genomes and RNA species released by human neoplastic cells. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 484-487.

256. Ebert, P.S. and Buell, D.N.: Viral reverse transcriptase suppression associated with erythroid differentiation of Friend leukemia cells. J Natl Cancer Inst 58: 635-640, 1977.

257. Ebert, P.S., Wars, I. and Buell, D.N.: Erythroid differentiation in cultured Friend leukemia cells treated with metabolic inhibitors. Cancer Res 36: 1809-1813, 1976.

258. Economou, C.K., Takeichi, N. and Boone, C.W.: Common tumor rejection antigens in methylcholanthrene-induced squamous cell carcinomas of mice detected by tumor protection and a radioisotopic footpad assay. Cancer Res 37: 37-41, 1976.

259. Edmondson, H.A., Henderson, B.E. and Benton, B.: Liver cell adenomas. N Engl J Med (In Press).

260. Edmondson, H.A., Reynolds, T.B., Henderson, B.E. and Benton, B.: Regression of liver cell adenomas associated with oral contracep-tives. Ann Intern Med (In Press).

261. Eichberg, J.W., McCullough, E., Kalter, S.S., Thor, D.E. and Rodriguez, A.R.: Clinical, virological and pathological features of herpesvirus SA8 infection in conventional and gnotobictic infant baboons (Papio cynccephalus). Archives of Virol (In Press).

262. Elder, J.H., Jensen, F.C., Bryant, M.L. and Lerner, R.A.: Polymorphism of the major envelope glycoprotein (gp70) of murine C-type viruses: Virion associated and differentiation antigens encoded by a multi-gene family. Nature (In Press).

263. Elder, J.H., Pickett, R.A., Hampton, J. and Lerner, R.A.: Radioiodination of proteins in single polyacrylamide gel slices: Tryptic peptide analysis of all the major members of complex multicomponent systems using microgram quantities of total protein. J Biol Chem (In Press).

264. Epstein, A.L., Herman, M., Kim, H., Dorfman, R.F. and Kaplan, H.S.: Biology of the human malignant lymphomas. III. Intracranial heterotransplantation in the nude, athymic mouse. Cancer 37: 2158-2176, 1976.

265. Epstein, R.S., Lopez, D.M., Ortiz-Muniz, G. and Sigel, M.M.: Emergence of a subpopulation of lymphocytes bearing theta antigen and complement receptor during tumor growth. Int J Cancer 18: 458-461, 1976.

266. Ernberg, I.: Chromosomal association of Epstein-Barr viral nuclear antigen by anti-complementary immunofluorescence. Adv Cell Res (In Press).

267. Esparza, J., Benyesh-Melnick, M. and Schaffer, P.A.: Intertypic complementation and recombination between temperature-sensitive mutants of herpes simplex virus types 1 and 2. Virology (In Press).

268. Essex, M., Cotter, S., Sliski, A.H., Hardy, W.D., Jr., Stephenson, J.R., Aaronson, S.A. and Jarrett, O.: Horizontal transmission of feline leukemia virus under natural conditions in a feline leukemia cluster household. Int J Cancer 19: 90-96, 1977.

269. Essex, M. and Lamon, E.W.: Host immune response to oncornavirus-induced tumors. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

270. Essex, M., Stephenson, J.R., Hardy, W.D., Jr., Cotter, S. and Aaronson, S.A.: Leukemia, lymphoma and fibrosarcoma of cats as models for similar diseases of man. In Hiatt, H.H., Watson, J.D. and Winston, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

271. Fabricant, R.N., DeLarco, J. and Todaro, G.J.: Nerve growth factor receptors on human melanoma cells in culture. Proc Natl Acad Sci USA 74: 565-569, 1977.

272. Falk, L.A., Deinhardt, F., Nonoyama, M., Schudel, A., Wolfe, L.G., Bergholz, C., Lapin, B., Yakoleva, L., Agrba, V., Henle, G. and Henle, W.: Herpesvirus papio: A lymphotropic herpesvirus of baboons that is related to Epstein-Barr virus. VIIIth Int Symp Comparative Leukemia Research.

273. Falk, L.A., Deinhardt, F., Nonoyama, M., Wolfe, L.G., Bergholz, C., Lapin, B., Yakoleva, L., Agrba, V., Henle, G. and Henle, W.: Properties of a baboon lymphotropic herpesvirus related to Epstein-Barr virus. Int J Cancer 18: 798-807, 1976.

274. Falk, L.A., Deinhardt, F., Wolfe, L.G., Johnson, D., Hilgers, J. and De Thé, G.: Epstein-Barr virus: Experimental infection of Callithrix jacchus marmosets. Int J Cancer 17: 785-788, 1976.

275. Falk, L.A., Henle, G., Henle, W., Deinhardt, F. and Schudel, A.: Transformation of lymphocytes by herpesvirus papio. Int J Cancer (In Press).

276. Falk, L.A., Schaffer, P.A., Wright, J., Deinhardt, F., Wolfe, L.G. and Benyesh-Melnick, M.: Experimental infection of marmoset and squirrel monkeys with attenuated HVS. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 336-338.

277. Farber, F.E. and Eberle, R.: Effects of cytochalasin and alkaloid drugs on the biological expression of herpes simplex virus type 2 DNA. Exp Cell Res 103: 15-22, 1976.

278. Fenyő, E.M. and Klein, E.: Diversity of murine C-type viruses. BBA, Reviews on Cancer (In Press).

279. Fenyő, E.M. and Klein, G.: Independence of Moloney virus-induced cell-surface antigen and membrane-associated viricn antigens in immunoselected lymphoma sublines. Nature 260: 355-357, 1976.

280. Ferdinand, F.-J., Brown, M. and Khoury, G.: Synthesis and character-ization of late lytic SV40 RNA from transcriptional complexes. Virology 1977 (In Press).

281. Feunteun, J., Sompayrac, L., Fluck, M.M. and Benjamin, T.L.: Localization of gene functions in polyoma virus DNA. Proc Natl Acad Sci USA 73: 4169-4173, 1976.

282. Fibach, E., Reuben, R.C., Rifkind, R.A. and Marks, P.A.: Effect of hexamethylene bisacetamide cn the commitment to differentiation of murine erythroleukemia cells. Cancer Res 37: 440-444, 1977.

283. Fine, D.L., Arthur, L.O. and Young, I.: Effect of temperature on mouse mammary tumor virus production. Infection & Immunity (In Press).

284. Fiori, M. and di Mayorca, G.: The occurrence of EKV DNA in DNA obtained from certain human tumors. Proc Natl Acad Sci USA 73(12): 4662-4666, 1976.

285. Flannery, V.L., Courtney, R.J. and Schaffer, P.A.: Expression of an early, nonstructural antigen of herpes simplex virus in cells transformed in vitro by herpes simplex virus. J Virol 21(1): 284-291, 1977.

286. Fluck, M.M., Staneloni, R.J. and Benjamin, T.L.: Hr-t and Ts-a: Two early gene functions of polyoma virus. Virology 1977 (In Press).

287. Fowler, A.K., Strickland, J.E., Kouttab, N.M. and Hellman, A.: RNA tumor virus expression in mouse uterine tissue during pregnancy. Biol Reprod 16: 344-348, 1977.

288. Fox, R.R., Diwan, B.A. and Meier, H.: Transplacental carcinogenic effects of combined ethylurea and sodium nitrite treatment in rabbits. J Natl Cancer Inst (In Press).

289. Frankel, A.E. and Fischinger, P.J.: Rate of divergence of cellular sequences homologous to segments of Moloney sarcoma virus. J Virol 21: 153-160, 1977.

290. Frankel, A.E. and Fischinger, P.J.: Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc Natl Acad Sci USA 73: 3705-3709, 1976.

291. Freeman, A.E., Lake, R.S., Igel, H.G., Gernand, L., Pezzutti, M.R., Malone, J.M., Marck, C. and Benedict, W.F.: Heteroploid conversion of human skin cells by methylcholanthrene. Proc Natl Acad Sci USA 1977 (In Press).

292. Frenkel, N., Locker, H., Cox, B., Roizman, B. and Rapp, F.: Herpes simplex virus DNA in transformed cells: Sequence complexity in 5 hamster cell lines and one derived hamster tumor. J Virol (In Press).

293. Friedman, R.M. and Costa, J.: The fate of interferon-treated cells. Infection & Immunity (In Press).

294. Friedman, R.M., Jay, F.T., Chang, E.H., Myers, M.W., Ramseur, J.M., Mims, S.J., Triche, T.J. and Wong, P.K.Y.: Interferon-directed inhibition of chronic murine leukemia virus production in cell cultures. J Infect Dis (In Press).

295. Friedmann, A. and Becker, Y.: Circular and circular-linear DNA molecules of herpes simplex virus. J Gen Virol 1977 (In Press).

296. Friedmann, A., Shlomai, J. and Becker, Y.: Electron microscopy of herpes simplex virus DNA molecules isolated from infected cells by centrifugation in CsCl density gradients. J Gen Virol 34: 507-522, 1977.

297. Friedrich, R., Morris, V.L., Goodman, H.M., Bishop, J.M. and Varmus, H.E.: Differences between genomes of two strains of mouse mammary tumor virus as shown by partial RNA sequence analysis. Virology 72: 330-340, 1976.

298. Friend, K., Hellström, I. and Hellström, K.E.: In vitro sensitization to embryonic antigens. Int J Cancer 18: 843-851, 1976.

299. Fuks, Z., Strober, S., Bobrove, A.M., Sasazuki, T., McMichael, A. and Kaplan, H.S.: Long term effects of radiation on T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. J Clin Investigation 58: 803-814, 1976.

300. Fuks, Z., Strober, S. and Kaplan, H.S.: Interaction between serum factors and T lymphocytes in Hodgkin's disease. N Engl J Med 295: 1273-1278, 1976.

301. Fuks, Z., Strober, S., King, D.P. and Kaplan, H.S.: Reversal of cell surface abnormalities of T lymphocytes in Hodgkin's disease after in vitro incubation in fetal sera. J Immunol 117: 1331-1335, 1976.

302. Funderburk, W.W. and Flax, R.L.: Surgical localization of occult mammography lesions. Breast, Diseases of the Breast (In Press).

303. Funderburk, W.W. and Flax, R.L.: Localization of nonpalpable carcinoma of the breast utilizing xeromammography. Breast, Diseases of the Breast 2: 28-29, 1976.

304. Furmanski, P., Loeckner, C.P., Longley, C., Larson, I. and Fich, M.A.: Identification and isolation of the major core protein from the oncornavirus-like particle in human milk. Cancer Res 36: 4001-4007, 1976.

305. Fuson, E.W. and Lamon, E.W.: IgM-induced cell-mediated cytotoxicity with antibody and effector cells of human origin. J Immunol 1977 (In Press).

306. Galehouse, D. and Duesberg, P.: RNA and proteins of the Kirsten sarcoma-xerotropic leukemia virus complex propagated in rat and duck cells. Virology 70(1): 97-104, 1976.

307. Galehouse, D. and Duesberg, P.: Differences in the glycoproteins of avian tumor virus recombinants: Evidence for intragenic crossing over. In Baltimore, D., Huang, A. and Fox, C.F. (Ed.): Animal Virology. New York, Academic Press, Inc., 1976, p 227-236.

308. Galili, N., Naor, D.A., Åsjö, B. and Klein, G.: Induction of immune responsiveness in a genetically low-response tumor-host combination by chemical modification of the immunogen. Eur J Immunol 6(7): 473-476, 1976.

309. Gallo, R.C., Gallagher, R.E., Gillespie, D.H., Saxinger, W.C., Wong-Staal, F., Reitz, M.S. and Smith, R.G.: Some thoughts on the etiology of human acute myelogenous leukemia. Cold Spring Harbor Symp, XL11 (In Press).

310. Gallo, R.C., Gillespie, D.H., Gallagher, R.E., Reitz, M.S., Miller, N.R. and Wong-Staal, F.: Some evidence for infectious type C virus in humans. Proc of the Fourth Annu ICN-UCLA Winter Conf on Molecular and Cellular Biology. New York, Academic Press, Inc. (In Press).

311. Gallo, R.C. and Reitz, M.S.: Molecular probes for tumor viruses in human neoplasia. Int Rev Exp Pathol 16: 1-58, 1976.

312. Gallo, R.C., Wong-Staal, F., Gillespie, D.H., Miller, N.R., Gallagher, R.E., Saxinger, W.C. and Reitz, M.S.: Evidence for the presence of type C virus in the human population. Proc of the Sixth Annu ICN-UCLA Symp on Molecular Biology. New York, Academic Press, Inc. (In Press).

313. Gardner, M.B.: Persistent infection of wild mice with murine leukemia virus. Microbiol Annu (In Press).

314. Gardner, M.B., Brown, J.C., Charman, H.P., Stephenson, J.R., Rongey, R.W., Hauser, D.E., Diegmann, F., Howard, E., Dworsky, R.I., Gilden, R.V. and Huebner, R.J.: FeLV epidemiology in Los Angeles cats: Appraisal of detection methods. Int J Cancer (In Press).

315. Gardner, M.B., Klement, V., Dougherty, M., McConahey, P., Estes, J.D. and Huebner, R.J.: Type C virus expression in lymphoma-paralysis prone LC wild mice. J Natl Cancer Inst (In Press).

316. Gardner, M.B., Klement, V., Estes, J.D., Gilden, R.V., Toni, R. and Huebner, R.J.: Suppression of infectious MuLV in wild mice by passive immunization. J Natl Cancer Inst (In Press).

317. Gardner, M.B., Klement, V., Henderson, B.E., Estes, J.D., Dougherty, M., Casagrande, J.C. and Huebner, R.J.: Efforts to control type C virus expression in wild mice. In Chirigos, M.A. (Ed.): Control of Neoplasia by Modulation of the Immune System. New York, Raven Press, 1977, p 391-407.

318. Gardner, M.B., Klement, V., Henderson, B.E., Estes, J.D., Dougherty, M., Casagrande, J.C. and Huebner, R.J.: Efforts to control type C virus expression in wild mice. Natl Cancer Inst Monogr (In Press).

319. Gardner, M.B., Klement, V., Rasheed, S., Rongey, R.W., Brown, J.C., Pike, M.C., Henderson, B.E. and Huebner, R.J.: The pathogenesis of lymphoma and paralysis in wild mice. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 204-208.

320. Gardner, M.B., Klement, V., Rongey, R.W., McConahey, P., Estes, J.D. and Huebner, R.J.: Type C virus expression in lymphoma-paralysis prone wild mice. J Natl Cancer Inst 57: 585-590, 1976.

321. Gardner, M.B., McAllister, R.M., Rasheed, S., Klement, V., Shimizu, S., Rongey, R.W., Charman, H.P., Gilden, R.V., Henderson, B.E., Heberling, R.L. and Huebner, R.J.: Search for RNA tumor virus in humans. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

322. Gardner, M.B., Rasheed, S., Klement, V., Rongey, R.W., Brown, J.C., Dworsky, R.L. and Henderson, B.E.: Lower motor neuron disease in wild mice caused by indigenous type C virus and search for a similar etiology in human ALS. In Andrews, J.M., Johnson, R.T. and Brazier, M.A.B. (Ed.): Amyotropic Lateral Sclerosis: Recent Research Trends. New York, Academic Press, Inc., 1976, p 217-234.

323. Geder, L.: Evidence for early nuclear antigens in cytomegalovirus-infected cells. J Gen Virol 32: 315-319, 1976.

324. Geder, L., Kreider, J. and Rapp, F.: Human cells transformed in vitro by human cytomegalovirus: Tumorigenicity in athymic nude mice. J Natl Cancer Inst 58: 1003-1007, 1977.

325. Geder, L. and Rapp, F.: Evidence for nuclear antigens in cytomeg-alovirus-transformed human cells. Nature 265: 184-186, 1977.

326. Gergely, P. and Ernberg, I.: Blastogenic response and EBNA induction in human lymphocytes by Epstein-Barr virus only requires B cells but not macrophages. Cancer Letters 2: 217-220, 1977.

327. Gershwin, M.E., Ikeda, R.M., Kawakami, T.G. and Owens, R.B.: Immunobiology of heterotransplanted human tumors in nude mice. J Natl Cancer Inst 1977 (In Press).

328. Geser, A., Charnay, N., Day, N.E., de Thé, G. and Ho, J.H.C.: Environmental factors in the etiology of nasopharyngeal carcinoma. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

329. Gilden, R.V.: Interrelationships among RNA tumor viruses and host cells. In Klein, G. and Weinhouse, S. (Ed.): Advances in Cancer Research. (In Press).

330. Gilead, Z., Jeng, Y.-H., Wold, W.S.M., Sugawara, K., Rho, H.M., Harter, M.L. and Green, M.: Immunological identification of two adenovirus 2-induced early proteins possibly involved in cell transformation. Nature 264(5583): 263-266, 1976.

331. Gilead, Z., Sugawara, K., Shanmugam, G. and Green, M.: Synthesis of the adenovirus coded DNA binding protein in infected cells. J Virol (In Press).

332. Gillette, R.W. and Boone, C.W.: Augmented immunogenicity of tumor cell membranes produced by surface budding viruses. Parameters of optical immunization. Int J Cancer 18: 212-222, 1976.

333. Ginsburg, C.M., Henle, G. and Henle, W.: An outbreak of infectious mononucleosis among the personnel of an outpatient clinic. Am J Epidemiol 104: 571-575, 1976.

334. Ginsburg, C.M., Henle, W., Henle, G. and Horwitz, C.A.: Infectious mononucleosis in children: Evaluation of the Epstein-Barr virus-specific serology. J Am Med Assoc 237: 781-785, 1976.

335. Glaser, R.: Expression and regulation of Epstein-Barr virus in mammalian cells. In Baltimore, D., Huang, A.S. and Fox, C.F. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976, p 641-658.

336. Glaser, R., Ablashi, D.V., Nonoyama, M., Henle, W. and Easton, J.M.: Enhanced oncogenic behavior of human and mouse cells following cellular hybridization with Burkitt tumor cells. Proc Natl Acad Sci USA (In Press).

337. Glaser, R., De Thé, G., Lenoir, G. and Ho, J.H.C.: Superinfection of epithelial nasopharyngeal carcinoma cells with Epstein-Barr virus. Proc Natl Acad Sci USA (In Press).

338. Glaser, R., Geder, L., St. Jeor, S.C., Michelson-Fiske, S. and Haguenau, F.: Partial characterization of a herpes-type virus (K9V) derived from Kaposi's sarcoma. J Natl Cancer Inst 1977 (In Press).

339. Glaser, R., Lenoir, G., Perrone, S., Pellegrino, M. and de Thé, G.: Cell surface markers on epithelial/Burkitt hybrid cells super-infected with Epstein-Barr virus. Cancer Res 1977 (In Press).

340. Goldberg, R.J. and Gravell, M.: A search for HSV-2 markers in cervical carcinoma. Cancer Res (In Press).

341. Goldblum, N., Ben-Bassat, H., Mitrani, S., Anderssor-Anvret, M., Goldblum, T., Aghai, E., Ramot, B. and Klein, G.: A case of an Epstein-Barr virus (EBV) genome-carrying lymphoma in an Israeli Arab child. Eur J Cancer 1977 (In Press).

342. Goodman, M.I., Levine, P.H. and Miller, D.: Nasopharyngeal carcinoma patients in Massachussetts. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

343. Gordon, R.J.: Distribution of airborne polycyclic aromatic hydrocarbons throughout Los Angeles. Environ Sci (In Press).

344. Gottlieb, J.A., Baker, L.H., O'Bryan, R.M., Sinkovics, J.G., Hoogstraten, B., et al: Adriamycin used alone and in combination in soft tissue and bony sarcomas. Cancer Chemother Rep (In Press).

345. Gravell, M., Levine, P.H., McIntyre, R.F., Land, V.J. and Pagano, J.S.: Epstein-Barr virus in an American patient with Burkitt's lymphoma: Detection of viral genome in tumor tissue and establishment of a tumor-derived cell line (NAE). J Natl Cancer Inst (In Press).

346. Green, M. and Mackey, J.K.: Are oncogenic human adenoviruses associated with human cancer? Analysis of human tumors for adenovirus transforming gene sequences. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

347. Green, M. and Wold, W.S.M.: Oncogenic DNA viruses - replication, tumor gene expression and role in human cancer. Seminars in Oncology (In Press).

348. Green, M.R., Chinnadurai, G., Mackey, J.K. and Green, M.: A unique pattern of integrated viral genes in hamster cells transformed by highly oncogenic human adenovirus 12. Cell (In Press).

349. Green, M.R., Mackey, J.K. and Green, M.: Multiple copies of human adenovirus 12 genomes are integrated in virus induced hamster tumors. J Virol 22 (In Press).

350. Greenberger, J.S., Bensinger, W.I. and Aaronson, S.A.: Selective techniques for the isolation of morphologic revertants of sarcoma virus transformed cells. In Prescott, D. (Ed.): Methods in Cell Biology 14. New York, Academic Press, Inc., p 237-248.

351. Greenlee, J.E., Narayan, O., Johnson, R.T. and Herndon, R.M.: Induction of brain tumors in hamsters with BK virus, a human papovavirus. Lab Invest (In Press).

352. Gross, L. and Schidlovsky, G.: The possible significance of the presence of C-type virus particles in placenta of normal healthy Sprague-Dawley rats. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

353. Gunvén, P.: Immunology of EBV infection. In Good, R.A. (Ed.): Immunology of Human Infection. New York, Plenum Press (In Press).

354. Gupta, P. and Rapp, F.: Effect of zinc ions on synthesis of herpesvirus type 2-induced polypeptides. Proc Soc Exp Biol Med 152: 455-458, 1976.

355. Gupta, P. and Rapp, F.: Identification of virion polypeptides in hamster cells transformed by herpes simplex virus type 1. Proc Natl Acad Sci USA 74: 372-374, 1977.

356. Gupta, P. and Rapp, F.: Effect of hormones on herpes simplex virus type-2 induced transformation. Nature 1977 (In Press).

357. Gupta, P., St. Jeor, S.C. and Rapp, F.: Comparison of the polypeptides of several strains of human cytomegalovirus. J Gen Virol 34: 447-454, 1977.

358. Haaijman, J.J.: Quantitative immunofluorescence microscopy; Methods and application. The Hague, TNO, 1977.

359. Hackett, A.J., Smith, H.S., Springer, E.L., Owens, R.B., Nelson-Rees, W.A., Riggs, J.L. and Gardner, M.B.: Two syrgeneic cell lines from the same human breast tissue: One aneuploid mammary epithelial (Hs578T) and one diploid myoepithelial (Hs578st). J Natl Cancer Inst (In Press).

360. Hackett, A.J. and Springer, E.L.: Scanning and transmission electron microscopy of human metastatic mammary carcinoma. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

361. Hampar, B., Aaronson, S.A., Derge, J.G., Chakrabarty, M., Showalter, S.D. and Dunn, C.Y.: Activation of an endogenous mouse type C virus by ultraviolet-irradiated herpes simplex virus types 1 and 2. Proc Natl Acad Sci USA (In Press).

362. Hampar, B., Watanaka, M., Aulakh, G., Derge, J.G., Lee, L. and Showalter, S.D.: Type C virus activation in "non-transformed" mouse cells by uv-irradiated herpes simplex virus. Virology 76: 876-881, 1977.

363. Hampar, B., Lenoir, G., Nonoyama, M., Derge, J.G. and Chang, S.-Y.: Cell cycle dependence for activation of Epstein-Barr virus by inhibitors of protein synthesis or medium deficient in arginine. Virology (In Press).

364. Hanisch, R., Dworsky, R.L., Henderson, B.E. and Gardner, M.B.: A search for clues to the etiology of amyotrophic lateral sclerosis. Arch Neurol 33: 456-457, 1976.

365. Harewood, K.R. and Ahmed, M.: Production of antiserum to the reverse transcriptase of Mason-Pfizer monkey virus. J Gen Virol (In Press).

366. Harrison, T.A., Barr, R.D., McCaffrey, R.P., Sarna, G., Silverstone, A.E., Perry, S. and Baltimore, D.: Terminal deoxyribonucleotidyl transferase in AKR leukemic cells and lack of relation of enzyme activity to cell cycle phase. Biochem Biophys Res Commun (In Press).

367. Hart, W.R., Townsend, D.E., Aldrich, J.O., Henderson, B.E., Roy, M. and Benton, B.: Histopathologic spectrum of vaginal adenosis and related changes in stilbestrol-exposed females. Cancer (In Press).

368. Hart, W.R., Zaharov, I., Kaplan, B.J., Townsend, D.E., Aldrich, J.O., Henderson, B.E., Roy, M. and Benton, B.: Cytologic findings in stilbestrol exposed females with emphasis on detection of vaginal adenosis. Acta Cytol 20: 7-14, 1976.

369. Harter, M.L., Shanmugam, G., Wold, W.S.M. and Green, M.: Detection of adenovirus type 2-induced early polypeptides using cycloheximide pretreatment to enhance viral protein synthesis. J Virol 19: 232-242, 1976.

370. Haseltine, W.A., Kleid, D.G., Panet, A., Rothenberg, E. and Baltimore, D.: Ordered transcription of RNA tumor virus genomes. J Mol Biol 106: 109-131, 1976.

371. Haseltine, W.A., Maxam, A. and Gilbert, W.: The Rous sarcoma virus is terminally redundant: The 5' sequence. Proc Natl Acad Sci USA 74: 1977.

372. Hashimoto, S.-I. and Green, M.: Multiple methylated cap sequences in adenovirus type 2 early mRNA. J Virol 20(2): 425-435, 1976.

373. Hatanaka, M.: Saturable and non-saturable process of sugar uptake: Effect of oncogenic transformation in transport and uptake of nutrients. J Cell Physiol 89: 745-749, 1976.

374. Hatanaka, M.: RNA dependent DNA synthesis (book chapter in Japanese). In Osawa, F. (Ed.): Genetic Information, Seibutsu Kagaku Koza III. Tokyo, Asakura-Shoten, 1976, p 86-114.

375. Hatanaka, M., Del Guidice, R. and Long, C.: A new quick screening of mycoplasma contamination in cell culture by adenine detection. Tissue Culture Assoc Manual 2: 437-438, 1976.

376. Hatanaka, M. and Long, C.: Alterations in pyrimidine uptake by mammalian cells infected with mycoplasma. Nature (In Press).

377. Hayman, E.G., Pal, B.K. and Roy-Burman, P.: RNA tumor virus phosphoproteins: Evidence for virus-specificity of phosphoryla-tion. J Gen Virol 1977 (In Press).

378. Hayman, M.J., Hunter, E. and Vogt, P.K.: Inhibition of avian sarcoma virus replication by glucosamine: A specific effect on the synthesis and processing of viral proteins. Virology (In Press).

379. Heberling, R.L., Kalter, S.S., Barker, S.T. and Weislow, O.S.: Isolation and biological properties of endogenous baboon (Papio cynocephalus) type C viruses. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

380. Heding, L., Olsen, R.G., Hoover, E.A., Yohn, D.S. and Schaller, J.P.: Preparation of cell free feline oncornavirus associated cell membrane antigen. J Natl Cancer Inst (In Press).

381. Heding, L., Schaller, J.P., Blakeslee, J.R. and Olsen, R.G.: Inactivation of tumor cell associated feline leukemia virus: A model for producing an infectious virus tumor cell vaccine. Cancer Res 36: 1647-1652, 1976.

382. Hehlmann, R.: RNA tumor viruses and human cancer. In Current Topics in Microbiology and Immunology. New York, Springer-Verlag, 1976, Vol 73, p 141-215.

383. Heimer, R. and Klein, G.: Circulating immune complexes in sera of patients with Burkitt's lymphoma and nasopharyngeal carcinoma. Int J Cancer 18: 310-316, 1976.

384. Heiniger, H.J., Huebner, R.J. and Meier, H.: Effect of allelic substitutions at the hairless locus on endogenous ecotropic murine leukemia virus titers and leukemogenesis. J Natl Cancer Inst 57: 1073-1074, 1976.

385. Hellman, A. and Fowler, A.K.: Type C RNA virus expression in reproductive tissue. Possible biological significance. Microbiology 1977 (In Press).

386. Hellman, A., Fowler, A.K., Strickland, J.E. and Kouttab, N.M.: A possible physiological function for type C RNA viruses. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 161-165.

387. Hellström, I. and Hellström, K.E.: Cell-mediated immunity to tumor antigens as studied in vitro. In Richards, M.A. (Ed.): Proc Am Philosophical Soc Symp, Philadelphia, Pa. (In Press).

388. Hellström, I. and Hellström, K.E.: Suppression of cell-mediated cytotoxicity by lymphocytes from tumor-bearing animals. Transplant Proc 8: 255-264, 1976.

389. Hellström, I. and Hellström, K.E.: Specific sensitization of lymphocytes to tumor antigens by co-cultivation with peritoneal cells exposed to such antigens. Int J Cancer 17: 748-754, 1976.

390. Hellström, I., Hellström, K.E. and Shantz, G.: Demonstration of tumor-associated immunity with a leukocyte adherence inhibition (LAI) assay. Int J Cancer 18: 354-361, 1976.

391. Hellström, K.E. and Hellström, I.: Cell-mediated immunity to mouse tumors. Some recent findings. Ann NY Acad Sci 276: 176-187, 1976.

392. Hellström, K.E. and Hellström, I.: Spontaneous tumor regression: Possible relationship to in vitro parameters of tumor immunity. J Natl Cancer Inst 44: 131-134, 1976.

393. Hellström, K.E. and Hellström, I.: Immunological enhancement of turmo growth. In Green, I., Cohen, S. and McCluskey, R.T. (Ed.): Mechanisms of Tumor Immunity. New York, J. Wiley and Sons, Inc., 1976, p 147-174.

394. Henderson, B.E., Benton, B., Cosgrove, M., Baptista, J., Aldrich, J.O., Townsend, D.E., Hart, W.R. and Mack, T.M.: Urogenital tract abnormalities in male offspring of women treated with diethylstilbestrol. Pediatrics 58: 505-507, 1976.

395. Henderson, B.E., Gerkins, V.R., Pike, M.C., Casagrande, J.C.: Endocrine function and breast cancer. Natl Cancer Inst Monogr (In Press).

396. Henderson, B.E., Louie, E., Jing, J., Buell, P. and Gardner, M.B.: Risk factors associated with nasopharyngeal carcinoma. N Engl J Med 295: 1101-1106, 1976.

397. Henderson, B.E., Pike, M.C., Gerkins, V.R. and Casagrande, J.C.: Hormonal basis of breast cancer: Elevated plasma levels of estrogen prolactin and progesterone. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

398. Henderson, G.B., Schrecker, A.W., Smith, C., Gordon, M., Zevely, E.M., Vitols, K.S. and Huennekens, F.M.: Transport of methotrexate and other folate compounds: Components, mechanism and regulation by cyclic nuclectides. Adv Enzyme Regul 1977 (In Press).

399. Henle, G.: Infectious mononucleosis. In: Epstein-Barr Virus Oncogenesis. Cancer Institute, Hokkaido University, Sapporo (In Press).

400. Henle, W.: Faktorenanalyse der Tumorentstehung beim Menschen am Beispiel des Epstein-Barr-Virus. Klin Wochenschr (In Press).

401. Henle, W.: The association of Epstein-Barr virus with Burkitt's lymphoma. In: Epstein-Barr Virus Oncogenesis. Cancer Institute, Hokkaido University, Sapporo (In Press).

402. Henle, W. and Henle, G.: Epstein-Barr virus-related serology in nasopharyngeal carcinoma and controls. In: The Etiology and Control of Nasopharyngeal Carcinoma. IARC Scientific Publications (In Press).

403. Henle, W. and Henle, G.: Evidence for an etiologic relation of the Epstein-Barr virus to human malignancies. Laryngoscope 87(4): 467-473, 1977.

404. Henle, W. and Henle, G.: Antibodies to the R component of EBV-induced early antigens in Burkitt's lymphoma may exceed in titer antibodies to EB viral capsid antigen. J Natl Cancer Inst 58: 785-786, 1977.

405. Henle, W. and Henle, G.: The seroepidemiology of Epstein-Barr virus. In Corek, G. and King, D.W. (Ed.): Herpesviruses, Advances in Pathobiology Series. New York, Intercontinental Med Book Corp., 1975 (In Press).

406. Hesse, J., Levine, P.H., Ebbesen, P., Connelly, R. and Mordhorst, C.H.: A case-control study on immunity to two Epstein-Barr virus-associated antigens and to herpes simplex virus and adenovirus in a population-based group of patients with Hodgkin's disease in Denmark, 1971-1973. Int J Cancer 19: 49-58, 1977.

407. Heston, W.E., Smith, B. and Parks, W.P.: Mouse mammary tumor virus in hybrids from strains C57E1 and GR - breeding test of backcross segregants. J Exp Med 144: 1022-1030, 1976.

408. Hilgers, J. and Bentvelzen, P.: Interaction between viral and genetic factors in murine mammary cancer: Possible implications for human disease. In Heuson, J.C., Mattheim, W.H. and Rozenweig, M. (Ed.): Breast Cancer: Trends in Research and Treatment. New York, Raven Press, 1976.

409. Hino, S., Stephenson, J.R. and Aaronson, S.A.: Radioimmunoassays for the 70,000 molecular weight glycoproteins of endogenous mouse type C viruses: Viral antigen expression in normal mouse tissues. J Virol 18: 933-941, 1976.

410. Hiraki, S., Mulcahy, M.P. and Dmochowski, L.: Particle-filament complex in tumor cells of northern pike, Esox lucius L. Tex Rep Biol Med (In Press).

411. Hirsch, M.S.: Immune activation of endogenous oncogenic viruses. Ann NY Acad Sci 276: 529-535, 1976.

412. Hirsch, M.S.: Immune activation of endogenous viruses. In Crowell, R., Friedman, H. and Prier, J. (Ed.): Tumor Virus Infections and Immunity. Baltimore, Md., University Park Press, 1976, p 175-186.

413. Hirsch, M.S.: Interactions between lymphocytes and C-type oncornaviruses in mice. Isr J Med Sci 12: 407-413, 1976.

414. Hirsch, M.S.: Interactions between C-type oncornaviruses and lymphocytes. In McCluskey, R. and Cohen, S. (Ed.): Mechanisms of Tumor Immunity. New York, John Wiley & Sons, Inc., 1977, p 287-303.

415. Hirsch, M.S., Proffitt, M.R. and Black, P.H.: Immunopathology and murine C-type virus infections. In Hanna, M.G. and Papp, P. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, Vol 6 (In Press).

416. Hirsch, M.S., Rinaldo, C.R., Jr. and Black, P.H.: Virus infections in bone marrow transplantation. Transplant Proc (In Press).

417. Ho, J.H.C.: Epidemiology of nasopharyngeal carcinoma. In Hirayama, T. (Ed.): Cancer in Asia. Baltimore, Md., University Press, 1976, p 49-61.

418. Ho, J.H.C., Chau, J.C.W. and Levine, P.H.: Longitudinal studies on delayed hypersensitivity reactions in nasopharyngeal carcinoma patients in Hong Kong. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

419. Ho, J.H.C., Ng, M.H. and Kwan, H.C.: Detection of saliva IgA antibodies to Epstein-Barr virus capsid antigens in nasopharyngeal carcinoma patients and controls. Br J Cancer (In Press).

420. Ho, J.H.C., Ng, M.H., Kwan, H.C. and Chau, J.C.W.: Epstein-Barr virus-specific IgA and IgG serum antibodies in nasopharyngeal carcinoma patients and controls. Br J Cancer 34: 655-660, 1976.

421. Hodgen, G.D., Wolfe, L.G., Ogden, J.D., Adams, M.R., Lescalzi, C.C. and Hildebrand, D.F.: Diagnosis of pregnancy in marmosets: Hemagglutination inhibition test and radioimmunoassay for urinary chorionic gonadotropin. Lab Anim Sci 26: 224-229, 1976.

422. Holben, J.A. and Moore, D.H.: Record-keeping for a colony of 15,000 mice without use of a computer. Lab Anim Sci 26: 600-602, 1976.

423. Holder, W.D., Jr., Peer, G.W., Bolognesi, D.P. and Wells, S.A., Jr.: Detection of the major glycoproteins of Friend leukemia virus (gp71) and the murine mammary tumor virus (gp52) on the surface of mouse cells. Cancer Res 36: 3217-3224, 1976.

424. Holland, J.F., Glidewell, O., Ellison, R.R., Schwartz, J., Wallace, H.J., Hoagland, C., Wiernik, P., Rai, K.K., Bekesi, J.G. and Cuttner, J.: Acute myelocytic leukemia. Arch Intern Med (In Press).

425. Hoover, E.A., Mathes, L.E., Schaller, J.P. and Olsen, R.G.: Horizontal transmission of feline leukemia virus under experimental conditions. J Natl Cancer Inst 58: 443-444, 1977.

426. Hoover, E.A., Olsen, R.G., Hardy, W.D., Jr., Schaller, J.P. and Mathes, L.E.: Feline leukemia virus infection: Age-related variation in responses of cats to experimental infection. J Natl Cancer Inst 57: 365-369, 1976.

427. Hoover, E.A., Olsen, R.G., Hardy, W.D., Jr., Schaller, J.P., Mathes, L.E. and Cockerell, G.L.: Responses of cats to infections with feline leukemia virus: Age-related factors and variations in disease caused by different virus strains. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 180-183.

428. Hoover, E.A., Schaller, J.P., Mathes, L.E. and Olsen, R.G.: Passive immunity to feline leukemia: Evaluation of immunity from dams naturally infected and experimentally vaccinated. Infection & Immunity 16 (In Press).

429. Horwitz, C.A., Burke, M.D., Henle, W., Henle, G., Penn, G. and Weinberg, D.: Later persistence of serum gamma glutamyl transpeptidase (GGT) activity following mononucleosis - report of three cases. Gastroenterology (In Press).

430. Horwitz, C.A., Henle, W., Henle, G., Polesky, H., Balfour, H.H., Jr., Siem, R.A., Borken, S. and Ward, P.: Heterophil-negative infectious mononucleosis and mononucleosis-like illnesses: Laboratory confirmation of 43 cases. Am J Med (In Press).

431. Horwitz, C.A., Henle, W., Henle, G., Segal, M., Arnold, T., Lewis, F.B., Zanick, D. and Ward, P.: Clinical and laboratory evaluation of elderly patients with heterophil-antibody positive infectious mononucleosis - report of seven patients, ages 40-78. Am J Med 61: 333-339, 1976.

432. Horwitz, C.A., Henle, W., Segal, M., Arnold, T., Lewis, F.B., Zanick, D. and Ward, P.: Clinical and laboratory evaluation of elderly patients with heterophil-antibody positive infectious mononucleosis - report of seven patients, ages 40-78. Am J Epidemiol (In Press).

433. Horwitz, C.A., Moulds, J., Henle, W., Henle, G., Polesky, H., Balfour, H.H., Jr. and Schwartz, B.: Cold agglutinins in infectious mononucleosis and heterophilantibody negative mononucleosis-like syndromes. Blood (In Press).

434. Howard, D.K., Colcher, D., Teramoto, Y.S., Young, J. and Schlom, J.: Characterization of mouse mammary tumor viruses propagated in non-murine cells. Cancer Res (In Press).

435. Howley, P., Khoury, G., Takemoto, K.K. and Martin, M.A.: Polynucleotide sequences common to the genomes of simian virus 40 and the human papovaviruses JC and BK. Virology 73: 303-307, 1976.

436. Hsu, H.W., Davis, D.S., Wei, C.H. and Yang, W.K.: Temperature effect on affinity chromatographic separation of two lectins from the seeds of Ricinus communis. Anal Biochem (In Press).

437. Hsu, I.C. and Yang, W.K.: DNA transfection ecotropic murine leukemia viruses in mouse culture cells. Cancer Res 37: 1977 (In Press).

438. Hu, S., Davidson, N., Nicolson, M.O. and McAllister, R.M.: A heter-oduplex study of the sequence relations between RD-114 and BKD RNA's. J Virol 1977 (In Press).

439. Hu, S., Davidson, N. and Verma, I.M.: A heteroduplex study of the sequence relationship between the RNA's of M-MSV and M-MLV. Cell 10: 469-478, 1977.

440. Huang, D.P., Ho, J.H.C., Lui, M. and Ng, M.H.: Growth stimulatory effects of Epstein-Barr virus nasopharyngeal mucosal explants. Br J Cancer (In Press).

441. Huang, E.-S. and Pagano, J.S.: Nucleic acid hybridization technology and detection of proviral genomes. In Koprowski, H. and Maramorosch, K. (Ed.): Methods in Virology. New York, Academic Press, Inc., 1977, 6: p 457-497.

442. Huebner, R.J.: The endogenous origin and transmission of RNA viral genomes that code for cancer. In Grundmann, E. (Ed.): Malignant Bone Tumors. Recent Results in Cancer Research Vol 54. Berlin, Springer-Verlag, 1976, p 63-91.

443. Huebner, R.J., Gilden, R.V., Lane, W.T., Toni, R., Trimmer, R.W. and Hill, P.R.: Suppression of type C RNA virogenes by type specific oncornavirus vaccine. Proc Natl Acad Sci USA (In Press).

444. Huebner, R.J., Gilden, R.V., Lane, W.T., Trimmer, R.W. and Hill, P.R.: Suppression of endogenous type C RNA virogene expression in mice by serotype-specific viral vaccines: Progress report. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 381-389.

445. Huebner, R.J., Gilden, R.V., Toni, R., Hill, P.R. and Trimmer, R.W.: Suppression of endogenous type C murine virogene expressions with virus specific hyperimmune immunoglobulin (IgG). In Nieburgs, H.E. (Ed.): 3rd Int Symp on Detection and Prevention of Cancer. New York, Marcel Dekker, Inc., Apr 1976 (In Press).

446. Huebner, R.J., Gilden, R.V., Toni, R., Hill, P.R., Trimmer, R.W., Fish, D.C. and Sass, B.: Prevention of spontaneous leukemia in AKR mice by type-specific immunosuppression of endogenous ecotropic virogenes. Proc Natl Acad Sci USA 73: 4633-4635, 1976.

447. Hunt, R.D., Blake, J.B., Daniel, M.D.: Herpesvirus saimiri lymphoma in owl monkeys (Aotus trivirgatus): Susceptibility, latent period, hematologic picture and course. Theriogenology 6: 139-152, 1976.

448. Hunter, E., Hayman, M.J., Rongey, R.W. and Vogt, P.K.: An avian sarcoma virus mutant which is temperature sensitive for virion assembly. Virology (In Press).

449. Hunter, E. and Vogt, P.K.: Temperature sensitive mutants of avian sarcoma viruses: Genetic recombination with wild type sarcoma virus and physiological analysis of multiple mutants. Virology (In Press).

450. Hunter, L. and Madin, S.H.: Clinical blood values of the northern fur seal, Callorhinus ursinus. J Wildlife Diseases 12: 526-530, 1976.

451. Hyman, R.W., Burke, S. and Kudler, L.: A nearby inverted repeat of the terminal sequence of herpes simplex virus DNA. Biochem Biophys Res Commun (In Press).

452. Hyman, R.W., Oakes, J.E. and Kudler, L.: In vitro repair of the preexisting nicks and gaps in herpes simplex virus DNA. Virology 76: 286-294, 1977.

453. Ibrahim, A., Ray, M., McGaw, J., Brown, E. and Nahmias, A.: Common antigens of herpes simplex virus 2 - associated hamster tumors and human cervical cancer. Proc Soc Exp Biol Med 152: 343-347, 1976.

454. Ihle, J.N., Collins, J.J., Lee, J.C., Fischinger, P.J., Moennig, V., Schäfer, W., Hanna, M.G., Jr. and Bolognesi, D.P.: Characterization of the immune response to the major glycoprotein (gp71) of Friend leukemia virus. I. Response in F/LE/c mice. Virology 75: 74-87, 1976.

455. Ihle, J.N., Collins, J.J., Lee, J.C., Fischinger, P.J., Pazmino, N.H., Moennig, V., Schäfer, W., Hanna, M.G., Jr. and Bolognesi, D.P.: Characterization of the immune response to the major glycoprotein (gp71) of Friend leukemia virus. III. Influence on endogenous MuLV-mediated pathogenesis. Virology 75: 102-111, 1976.

456. Ihle, J.N., Lee, J.C., Collins, J.J., Fischinger, P.J., Pazmino, N.H., Moennig, V., Schäfer, W., Hanna, M.G., Jr. and Bolognesi, D.P.: Characterization of the immune response to the major glycoprotein (gp71) of Friend leukemia virus. II. Response in C57BL/6 mice. Virology 75: 88-101, 1976.

457. Imamura, M., Mellors, R.C., Strand, M. and August, J.T.: Murine type C viral envelope glycoprotein gp69/71 and Lupus-like glomerulonephritis of New Zealand mice. An immunoperoxidase study. Am J Pathol 86: 375-386, 1977.

458. Ishizaki, R., Green, R.W. and Bolognesi, D.P.: The structural polypeptides of equine infectious anemia virus. Intervirology (In Press).

459. Ito, K., Arens, M. and Green, M.: Characterization of DNA polymerase associated with nuclear membrane fractions from uninfected and Ad2 infected KB cells. Biochim Biophys Acta 447: 340, 1976.

460. Iwai, H., Parker, J.C., Quist, K.E. and Van Hoosier, G.L., Jr.: Serologic reactivity to paramyxoviruses in guinea pig serum. Lab Anim Sci (In Press).

461. Jacobs, R.F., Aurelian, L. and Cole, G.A.: Cell-mediated immune response to herpes simplex virus: Type specific lymphoproliferative responses in lymph nodes draining the site of primary infection. J Immunol (In Press).

462. Jamjoom, G.A., Naso, R.B. and Arlinghaus, R.B.: A selective decrease in the rate of cleavage of an intracellular precursor to Rauscher leukemia virus p30 by treatment of infected cells with actinomycin D. J Virol 19: 743-745, 1976.

463. Jamjoom, G.A., Naso, R.B. and Arlinghaus, R.B.: Further characterization of intracellular precursor polyproteins of Rauscher leukemia virus. Virology 78: 1977 (In Press).

464. Jeng, Y.-H., Wold, W.S.M., Sugawara, K., Gilead, Z. and Green, M.: Adenovirus type 2 coded single stranded DNA binding protein: In vivo phosphorylation and modification. J Virol (In Press).

465. Jensen, F.C., Welsh, R.M., Cooper, N.R. and Oldstone, M.B.A.: Lysis of oncornaviruses by human serum. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 438-440.

466. Jenson, A.B., Groff, D.E., McConahey, P.J. and Dixon, F.J.: Transmission of murine leukemia virus (Scripps) from parent to progency mice: A comparison of assay systems. J Natl Cancer Inst 57(2): 421-424, 1976.

467. Jenson, A.B., Groff, D.E., McConahey, P.J. and Dixon, F.J.: Vertical transmission of murine leukemia virus (Scripps) in mice as determined by p30 antigenemia. Cancer Res (In Press).

468. Jing, J., Louie, E., Henderson, B.E. and Terasaki, P.I.: HLA patterns in nasopharyngeal carcinoma cases from California. In Henderson, B.E. (Ed.): Proc Symp on Epidemiology and Cancer Registries in the Pacific Basin, Maui, Hawaii, Nov 1975. Los Angeles, Ca., USC Press (In Press).

469. Jing, J., Louie, E., Henderson, B.E. and Terasaki, P.I.: HLA patterns in nasopharyngeal carcinoma cases from California. Natl Cancer Inst Monogr (In Press).

470. Johnson, R.T., Weiner, L.P., Narayan, O. and Greenlee, J.E.: Progressive multifocal leukoencephalopathy. In ter Meulen, V. and Katz, M. (Ed.): Slow Virus Infections of the Central Nervous System. (In Press).

471. Johnson, R.W., Benton, C.V., Perry, A., Batgi, J. and Shibley, G.P.: Contamination monitoring and control in a large scale tissue culture virus production laboratory. In Actin, R. and Lynn, D. (Ed.): Proc 1st Biannu Cell Culture Congress, Birmingham, Ala., Univ of Alabama Press, 1975 (In Press).

472. Jondal, M.: Spontaneous lymphocyte-mediated cytotoxicity (SIMC) in vitro: Assay for, and removal of the effector cell. In Bloom and David (Ed.): In Vitro Methods in Cell-Mediated Immunology. (In Press).

473. Jondal, M.: Isolation of human T lymphocytes from tumor tissues. In Bloom and David (Ed.): In Vitro Methods in Cell-Mediated Immunology. (In Press).

474. Jondal, M.: Antibody-dependent cellular cytotoxicity (ADCC) against Epstein-Barr virus-determined membrane antigens. I. Reactivity in sera from normal persons and from patients with actue infectious mononucleosis. Clin Exp Immunol 25: 1-5, 1976.

475. Jondal, M.: Assays for EBV receptors. In Bloom and David (Ed.): In Vitro Methods in Cell-Mediated Immunology. (In Press).

476. Jondal, M.: SRBC rosette formation as a human T lymphocyte marker. Scand J Immunol 5(5): 69-76, 1976.

477. Jondal, M., Klein, G., Oldstone, M.B.A., Bokish, V. and Yefenof, E.: Surface markers on human B and T lymphocytes. VIII. Association between complement and Epstein-Barr virus receptors on human lymphoid cells. Scand J Immunol 5: 401-410, 1976.

478. Jondal, M., Svedmyr, E., Klein, E. and Klein, G.: Epstein-Barr virus (EBV) specific T and K cell cytotoxicity. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 265-269.

479. Joseph, D.R., Kenney, F.T. and Farrelly, J.G.: Separation of cellular and viral DNA polymerases by affinity chromatography on polynucleotide-sepharose. Biochem Biophys Res Commun (In Press).

480. Junghans, R.E., Hu, S., Knight, C.A. and Davidson, N.: Heteroduplex analysis of avian RNA tumor viruses, I. Proc Natl Acad Sci USA 74: 477-481, 1977.

481. Kaaden, O.R., Scholz, A., Ben-Zeev, A. and Becker, Y.: Isolation of Marek's disease virus DNA from infected cells by electrophoresis on polyacrylamide gels. Arch Virol 53: 1977 (In Press).

482. Kacian, D.L. and Myers, J.C.: Synthesis of extensive, possibly complete, DNA copies of poliovirus RNA in high yields and at high specific activities. Proc Natl Acad Sci USA 73(7): 2191-2195, 1976.

483. Kacian, D.L. and Myers, J.C.: Anticomplementary nature of smaller DNA produced during synthesis of extensive DNA copies of poliovirus RNA. Proc Natl Acad Sci USA 73: 3408-3412, 1976.

484. Kai, K., Ikeda, H., Yuasa, Y., Suzuki, S. and Odaka, T.: Mouse strain resistant to N-, B-, and NB-tropic murine leukemia viruses. J Virol 20: 436-440, 1976.

485. Kaji, A., Yoshimura, M., Mutc, M., Chi, J., Roth, S.I., Delatto, R. and Okayama, M.: Differentiation and cellular transformation: An in vitro experiment using a temperature sensitive Rous sarcoma virus mutant. Cancer Chemother Rep 4(supplement 3): 1977.

486. Kakefuda, T., Lovinger, G.G., Gilden, R.V. and Hatanaka, M.: Electron microscopic studies of circular DNA in mouse embryo fibroblasts infected by Rauscher leukemia virus. J Virol 21: 792-795, 1977.

487. Kall, M.A., Hellström, I. and Hellström, K.E.: In vitro generation of primary and secondary cytotoxic cell-mediated immune responses to chemically induced mouse sarcomas. Int J Cancer 18: 488-497, 1976.

488. Kalter, S.S., Ablashi, D.V., Espana, C., Heberling, R.L., Hull, R.N., Lennette, E.H., Malherbe, H.H., McConnell, S., Pereira, H. and Yohn, D.S.: A review of simian virus nomenclature with suggested changes. Intervirology (In Press).

489. Kalter, S.S. and Heberling, R.L.: Herpesvirus infections of nonhuman primates. In Proc XX World Veterinary Congress, Jul 6-12, 1975, Thessaloniki, Greece (In Press).

490. Kalter, S.S., Heberling, R.L. and Malherbe, H.H.: Problems associated with nonhuman primates in biomedical research. In Proc VI Symposium of the ICLA, Thessaloniki, Greece, Jul 5-11, 1975. Stuttgart, Gustav Fischer Verlag (In Press).

491. Kalter, S.S., Kniker, W.T., Harvey, J.W., Jr., Pelsburg, P.J. and Heberling, R.L.: Lymphocyte responsiveness in squirrel, cebus and marmoset monkeys infected with H. saimiri. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

492. Kang, C.-Y. and Lanbright, P.: Pseudotypes of vesicular stomatitis virus with the mixed coat of reticuloendotheliosis virus and vesicular stomatitis virus. J Virol 21: 1252-1255, 1977.

493. Kaplan, H.S.: Hodgkin's disease and other human malignant lymphomas: Advances and prospects--G.H.A. Clowes Memorial Lecture. Cancer Res 36: 3863-3878, 1976.

494. Kaplan, H.S. and Gartner, S.: "Sternberg-Reed" giant cells of Hodgkin's disease: Cultivation in vitro, heterotransplantation, and characterization as neoplastic macrophages. Int J Cancer 1977 (In Press).

495. Kaplan, J. and Peterson, W.D., Jr.: Detection of T-cell lymphomaassociated antigens on cord blood lymphocytes and phytohemag-glutinin-stimulated blasts. Cancer Res 36: 3471-3475, 1976.

496. Kaplan, J., Ravindranath, Y. and Peterson, W.D., Jr.: T and B lymphocyte antigen-positive null cell leukemias. Blood 49(3): 371-378, 1977.

497. Kaplan, J., Tilton, J. and Peterson, W.D., Jr.: Identification of T cell lymphoma tumor antigens on human T cell lines. Am J Hematol 1: 219-223, 1976.

498. Kaplan, M.M., Lubiniecki, A.S., Blattner, W.A., Mason, T., Giard, D.J., Gunnell, M., Triantifellu, N. and Fraumeni, J.F.: Systematic variables affecting SV40-induced T-antigen expression and trans-formation in human cells. J Clin Microbiol 3: 593-598, 1976.

499. Kashmiri, S.V.S., Rein, A., Bassin, R.H., Gerwin, B.I. and Gisselbrecht, S.: Donation of N- or B-tropic phenotype to NB-tropic MuLV during mixed infections. J Virol 1977 (In Press).

500. Katz, A.D. and Henderson, B.E.: Parotid tumors in family groups and selected epidemiological observations. Arch Otolaryngol (In Press).

501. Kawakami, T.G., McDowell, T.S., Johnson, D.L., Brezrock, A.W. and Harrold, J.B.: Oncornavirus-host interaction in gibbons. In Clemmesen, J. and Yohn, I.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

502. Kay, H.D., Thota, H. and Sinkovics, J.G.: A comparative study on in vitro cytotoxic reactions of lymphocytes from normal donors and patients with sarcomas to cultured tumor cells. Clin Immunol Immunolpathol (In Press).

503. Keller, P.W., Hierholzer, J.C., Rubin, F.H., Black, P.H. and Hirsch, M.S.: Isolation of adenovirus type 34 from a renal transplant recipient with interstitial pneumonia. Transplantation 23: 188-191, 1977.

504. Kelloff, G.J., Peters, R.L., Donahoe, R.M., Stephenson, J.R. and Aaronson, S.A.: Natural occurrence of tumors in mouse strains differing in their xenotropic and ecotropic endogenous viruses. J Natl Cancer Inst 57: 85-89, 1976.

505. Kenney, F.T., Lane, S.E., Lee, K.-L. and Ihle, J.N.: Glucocorticoid control of gene expression. In Criss, W.E., Ono, T. and Sabine, J.R. (Ed.): Proc VII Int Symp on Modified Cellular and Molecular Controls in Neoplasia, Dec 1975, Honolulu, Hawaii. New York, Raven Press, 1976 (In Press).

506. Keydar, E., Unger, E., Galili, N., Klein, G., Åsjö, E., Bonavida, B. and Naor, D.A.: Immunogenicity of tumor cells modified by trinitrobenzene sulfonic acid (TNBS). J Supramol Struct (In Press).

507. Khoury, G. and Croce, C.M.: Assignment of the integrated SV40 DNA to human chromosome 7 in a SV40-transformed human cell line. Cytogenet. Cell Genet 16: 164-170, 1976.

508. Khoury, G., Howley, P., Israel, M., Brown, D. and Lai, C.-J.: The papovaviruses: Their molecular biology and potential etiology in human disease. In Allison, F., Jr. and Rothschild, H. (Ed.): Human Diseases Caused by Cancer: Recent Developments. New Orleans, La., Oxford University Press, 1977 (In Press).

509. Khoury, G., Lai, C.-J., Brown, D., Israel, M. and Howley, P.: The human papovaviruses and their potential role in human disease. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

510. Khoury, G. and May, E.: Regulation of early and late SV40 trans-cription: Dependence of late RNA synthesis of a functional T-antigen. J Virol 1977 (In Press).

511. King, N.W., Barahona, H., Daniel, M.D., Bekesi, J.G. and Jones, T.C.: The effect of phosphonoacetic acid on the ultrastructure of cell cultures infected with simian oncogenic herpesviruses. Lab Invest 1977 (In Press).

512. Kiuchi, M. and Takasugi, M.: The nonselective cytotoxic cell (N cell). J Natl Cancer Inst (In Press).

513. Klein, D.L. and Pearson, J.W.: Modulation of host immune response against the L2C guinea pig leukemia. Fed Proc (In Press).

514. Klein, E.: Burkitt lymphoma (BL), virus induced malignancy? Front Matrix Biol 3: 1-14, 1976.

515. Klein, E., Becker, S., Svedmyr, E., Jondal, M. and Vanky, F.: Tumor infiltrating lymphocytes. Ann NY Acad Sci 276: 207-216, 1976.

516. Klein, G.: The Epstein-Barr virus and human malignancies. In Borek, C. and West King, D. (Ed.): Advances in Pathology, 5. Cancer Biology III. Herpes Virus, Epidemiology, Molecular Events. Oncogenecity and Therapy. New York, Stratton Intercontinental Medical Book Corp., 1976, p 18-24.

517. Klein, G.: Immune surveillance - A powerful mechanism with a limited range. Natl Cancer Inst Monogr 44: 109-113, 1976.

518. Klein, G.: Tumor associated antigens in H-2 hemizygous isoantigenic variants of a somatic cell hybrid, derived from the fusion of a methylcholanthrene induced sarcoma and a mammary carcinoma. J Natl Cancer Inst (In Press).

519. Klein, G., Clements, G., Zeuthen, J. and Westman, A.: Somatic cell hybrids between human lymphoma lines. II. Spontaneous and induced patterns of the Epstein-Barr virus (EBV) cycle. Int J Cancer 17: 715-724, 1976.

520. Klein, G., Clements, G., Zeuthen, J. and Westman, A.: Spontaneous and induced patterns of the Epstein-Barr virus (EBV) cycle in a new set of somatic cell hybrids. Cancer Letters (In Press).

521. Klein, G. and Klein, E.: Immune surveillance against virus induced tumors and non-rejectability of spontaneous tumors: Contrasting consequences of host versus tumor evolution. Proc Natl Acad Sci USA (In Press).

522. Klein, G. and Klein, E.: Rejectability of virus induced tumors and non-rejectability of spontaneous tumors - a lesson in contrasts. Transplant Proc (In Press).

523. Klein, G., Terasaki, P.I., Billing, R.J., Honig, R., Jondal, M., Rosén, A., Zeuthen, J. and Clements, G.: Somatic cell hybrids between human lymphoma lines. III. Surface markers. Int J Cancer 19: 66-76, 1977.

524. Klein, G., Zeuthen, J., Terasaki, P.I., Billing, R.J., Honig, R., Jondal, M., Westman, A. and Clements, G.: Inducibility of the Epstein-Barr virus (EBV) cycle and surface marker properties of EBV-negative lymphoma lines and their in vitro EBV-converted sublines. Int J Cancer 18: 639-652, 1976.

525. Klein, J., Merryman, C.F., Maurer, P.H., Hauptfeld, M. and Gardner, M.B.: Histocompatability-2 system of wild mice. IV. Ia and Ir typing of two wild mouse populations. In Hiatt, H.E., Watson, J.D. and Winston, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

526. Klement, V., Gardner, M.B., Henderson, B.E., Ihle, J.N., Estes, J.D., Stanley, A.G. and Gilden, R.V.: Inefficient humoral immune respone of lymphoma-prone wild mice to persistent leukemia virus infection. J Natl Cancer Inst 57: 1169-1173, 1976.

527. Klement, V. and Nicolson, M.C.: Methods for assays of RNA tumor viruses. In Methods in Virology, Vol VI (In Press).

528. Kohl, S., Starr, S.E., Oleske, J.M., Shore, S., Ashman, R. and Nahmias, A.: Human monocyte-macrophage mediated antibodydependent cytotoxicity to herpes simplex virus infected cells. J Immunol 118: 729-735, 1977.

529. Koide, Y. and Takasugi, M.: Distinct target determinants on two lymphoblastoid lines derived from the same individual. J Immunol 117: 1197-1202, 1976.

530. Kouri, R.E.: Relationship between levels of aryl hydrocarbon hydroxylase activity and susceptibility to 3-methylcholanthrene and benzo(a)pyrene-induced cancers in inbred strains of mice. In Freudenthal, R.I. and Jones, P.W. (Ed.): Carcinogenesis, Vol 1. Polynuclear Aromatic Hydrocarbons: Chemistry, Metabolism, and Carcinogenesis. New York, Raven Press, 1976, p 139-151.

531. Kouri, R.E.: The role of the enzyme complex, aryl hydrocarbon hydroxylase (AHH), in chemically-induced cancers. In Freudenthal, R.I. and Jones, P.W. (Ed.): Polynuclear Aromatic Hydrocarbons; Chemistry, Metabolism, and Carcinogenesis. New York, Raven Press (In Press).

532. Kouri, R.E. and Nebert, D.W.: Genetic regulation of susceptibility to polycyclic hydrocarbon-induced tumors in the mouse. In Hiatt, H.H., Watson, J.D. and Winston, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

533. Kouttab, N.M., Fowler, A.K., Strickland, J.E. and Hellman, A.: Suppression of in vitro lymphocyte stimulation in mice by uterine and placental extracts. J Immunol 117: 1644-1650, 1976.

534. Krakower, J.M., Barbacid, M. and Aaronson, S.A.: Radioimmunoassay for mammalian type C viral reverse transcriptase. J Virol (In Press).

535. Krakowka, S.G., Olsen, R.G. and Cockerell, G.L.: Methods for producing T and B lymphocyte to receptor-specific antisera. J Immunol Method 14: 257-262, 1977.

536. Krakowka, S.G., Olsen, R.G. and Cockerell, G.L.: The effect of cell synchronization upon the detection of T and B lymphoid cell receptors on continuous lymphoid cell lines. In Vitro (In Press).

537. Krantz, M., Strand, M. and August, J.T.: Biochemical and immunological characterization of major envelope glycoproteins and degradation fragments from Rauscher murine virus. J Virol (In Press).

538. Krueger, G.R., Samii, H., Sesterhenn, K., Uhlmann, C.E., Ablashi, D.V., Fischer, R. and Wustrow, F.: Non-Hodgkin lymphomas: Cell population and functional behavior. In Ghierfelder, S., Rodt, E. and Thid, E. (Ed.): Immunological Diagnosis of Leukemia and Lymphomas. Munich, J.F. Lehmanns, Verlag (In Press).

539. Kung, H.J., Hu, S., Bender, W., Bailey, J.M., Davidson, N., Nicolson, M.O. and McAllister, R.M.: RD-114, baboon and woolly monkey viral RNA's compared in size and structure. Cell 1975 (In Press).

540. Kung, P.C., Gottlieb, P.D. and Baltimore, D.: Terminal deoxynucleotidyl transferase: Serological studies and radioimmunoassay. J Biol Chem (In Press).

541. Kurth, R., Bosch, V. and Bolognesi, D.P.: Polypeptides of endogenous avian C-type viruses. I. Their detection in the plasma membrane of normal and infected cells. Virology (In Press).

542. Laing, C.A., Heppner, G.H. and Bekesi, J.G.: Tumor antigens: Biological activity of components of soluble antigens of MTVinduced mammary tumors. Cancer Immunol and Immunotherapy 1977 (In Press).

543. Lamelin, J.P., Revillard, J.P., Chalopin, J.M., Ho, D.H.C., Souissi, T., Schwaab, G. and de Thé, G.: Cold lymphocytotoxic antibodies in nasopharyngeal carcinoma. Br J Cancer (In Press).

544. Lamelin, J.P., Revillard, J.P., Gabbiani, G. and de Thé, G.: Cold lymphocytotoxins and antiactin antibodies in nasopharyngeal carcinoma. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

545. Lamelin, J.P., Williams, E.H., Souissi, T., de Thé, G. and Gabbiani, G.: Smooth-muscle antibody in Burkitt's lymphoma and in nasopharyngeal carcinoma. Clin Exp Immunol (In Press).

546. Lamon, E.W.: Stimulation of tumor cell growth in vitro: A critical evaluation of immunologic specificity. J Natl Cancer Inst 1977 (In Press).

547. Lamon, E.W., Andersson, B., Whitten, H.D., Hurst, M.M. and Ghanta, V.: IgM complex receptors on subpopulations of murine lymphocytes. J Immunol (In Press).

548. Lamon, E.W., Hale, P. and Whitten, H.D.: Antibody dependent cell mediated cytotoxicity with autochthonous lymphocytes and sera following Moloney sarcoma virus infection. J Natl Cancer Inst (In Press).

549. Lamon, E.W., Shaw, M.W., Goodson, S., Lidin, P., Walia, A.S. and Fuson, E.W.: Antibody-dependent cell-mediated cytotoxicity in the Moloney sarcoma virus system: Differential activity of IgG and IgM with different subpopulations of lymphocytes. J Exp Med 145: 302-313, 1977.

550. Lange, B.J., Berman, P.H., Jr., Bender, J., Henle, W. and Bewetson, J.F.: Encephalitis in infectious mononucleosis: Diagnostic considerations. Pediatrics 58: 877-880, 1976.

551. Lasfargues, E.Y. and Sheffield, J.B.: Infectivity and onco-genicity of the mouse mammary tumor virus in tissue culture. J Microscopie et Biol, Cell 26: 29-34, 1976.

552. Lasfargues, E.Y., Vaidya, A.B., Lasfargues, J.C. and Moore, D.H.: Brief Communication: In vitro susceptibility of mink lung cells to the mouse mammary tumor virus. J Natl Cancer Inst 57: 447-449, 1976.

553. Lausch, R.N., Murasko, D.M. and Hay, K.A.: Tumor cell survival in a hostile immune environment. In Weiss, L. (Ed.): Fundamental Aspects of Metastasis. North-Holland Publishing Company (In Press).

554. Lausch, R.N. and Rapp, F.: Surface antigens on human herpesvirus-infected and -transformed cells. In Nicolau, C. (Ed.): Virus-transformed Cell Membranes. New York, Academic Press, Inc., 1977 (In Press).

555. Ledbetter, J., Nowinski, R.C. and Emery, S.: Viral proteins expressed on the surface of murine leukemia cells. J Virol (In Press).

556. Lee, J.C., Ihle, J.N. and Huebner, R.J.: The humoral immune response of NIH Swiss and SWR/J mice to vaccination with formalinized AKR or Gross murine leukemia virus. Proc Natl Acad Sci USA 74: 343-347, 1977.

557. Lejneva, O.M., Abelev, G.I., Dorfman, N.A., Strand, M. and August, J.T.: Localization of a murine oncornavirus 15,000 dalton virion protein on the membrane of neoplastic cells: Analysis by immunofluorescence and immunoelectron microscopy. Virology 75: 281-292, 1976.

558. Lemons, R., O'Brien, S. and Sherr, C.J.: A new genetic locus, Bevi, on human chromosome six which controls the replication of baboon type C virus in human cells. Cell (In Press).

559. Lenoir, G., Martin-Berthelon, M.C., Favre, M.C. and De Thé, G.: Characterization of EBV antigens I - Biochemical analysis of the complement-fixing soluble artigen and relationship with EBNA. J Virol (In Press).

560. Leong, J.C., Smith, H.S. and Goodman, H.M.: Viral RNA in the cytoplasm of SV40-transformed mouse cells: Post-translational processing. Virology (In Press).

561. Levin, R., Ruscetti, S.K., Parks, W.P. and Scolnick, E.M.: Expression of feline type C virus in normal and tumor tissues of the domestic cat. Int J Cancer 18: 661-671, 1976.

562. Levine, P.H.: Is Burkitt's lymphoma one disease? Conf on Early Life in Man and Animals, Perugia Quadrennial Int Conf on Cancer, Perugia, Italy, Jun 1977 (In Press).

563. Levine, P.H.: Clinical implications of immunity to oncogenic viruses. In Rapp, F. and Hanna, M.G. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, 1977 (In Press).

564. Levine, P.H., Connelly, R., Heath, C., Caldwell, G.G., Terasaki, P.I., Mickey, M.R. and Fabrizio, P.: An evaluation of antibody titers to the Epstein-Barr virus as a test for susceptibility to cancer. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

565. Levine, P.H., Stevens, D.A. and Lamellin, J.P.: Cellular immunity studies on NPC patients and controls. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

566. Levy, J.A.: Endogenous C-type viruses: Double agents in natural life processes. Biomedicine 24: 84-93, 1976.

567. Li, J.H. and Rapp, F.: Oncogenic transformation of mammalian cells in vitro by proflavine-photoinactivated herpes simplex virus type 2. Cancer Letters 1: 319-326, 1976.

568. Lieber, M.M., Smith, B., Szakal, A., Nelson-Rees, W.A. and Todaro, G.J.: A continuous cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int J Cancer (In Press).

569. Livingston, D.M., Howard, T. and Spence, C.: Identification of infectious virions which are vesicular stomatitis virus pseudotypes of murine type C virus. Virology (In Press).

570. Long, J.C., Aisenberg, A.C. and Zamecnik, P.C.: Chromatographic and electrophoretic analysis of an antigen in Hodgkin's disease tissue cultures. J Natl Cancer Inst 58: 223-227, 1977.

571. Lopez, D.M., Ortiz-Muniz, G. and Sigel, M.M.: Macrophage migration inhibition and lymphocyte stimulation with mammary tumor virusassociated antigens in BALB/c mice. Proc Soc Exp Biol Med 151: 225-230, 1976.

572. Lopez, D.M. and Sigel, M.M.: Tumor antigen extracts stimulate cytotoxicity against unrelated target cells. Int J Cancer 18: 808-812, 1976.

573. Lopez, D.M. and Sigel, M.M.: Correlation of tumor growth and cell-mediated immune responses of mice bearing mammary tumors following surgical procedures. J Reticulendothelial Soc 18(5): 305-312, 1975.

574. Louie, E., Henderson, B.E., Jing, J. and Buell, P.: A case-control study of nasopharyngeal carcinomas in California. Natl Cancer Inst Mcnogr (In Press).

575. Louie, E., Henderson, B.E., Jing, J. and Buell, P.: A case-control study of nasopharyngeal carcinoma in California. In Henderson, B.E. (Ed.): Proc Symp on Epidemiology and Cancer Registries in the Pacific Basin, Maui, Hawaii, Nov 1975. Los Angeles, Ca., USC Press (In Press).

576. Lubiniecki, A.S.: Target theory applied to acute leukemia in Down's syndrome and the general population. In Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F. (Ed.): Genetics of Human Cancer. New York, Raven Press, 1977, p 83-85.

577. Lubiniecki, A.S., Blattner, W.A., Dosik, H., Sun, C. and Fraumeni, J.F.: SV40-antigen expression in skin fibroblasts from clinically normal individuals and from ten cases of Fanconi's anemia. Am J Hematol 2: 33-40, 1977.

578. Lubiniecki, A.S., Blattner, W.A. and Fraumeni, J.F.: Elevated expression of SV40 T-antigen in simian papovavirus 40-infected skin fibroblasts from individuals with cytogenetic defects. Cancer Res (In Press).

579. Lubiniecki, A.S., Blattner, W.A. and Fraumeni, J.F.: SV40 T-antigen expression in skin fibroblast from normal individuals patients with Fanconi's anemia, and a family at high risk of leukemia. In Mulvihill, J.J., Miller, R.W. and Fraumeni, J.F., Jr. (Ed.): Genetics of Human Cancer. New York, Raven Press, 1977, p 377-380.

580. Lubiniecki, A.S., Costa, J. and Rabson, A.S.: Elevated expression of T-antigen in skin fibroblasts from individuals with cytogenetic anomalies infected in vitro with simian or human papovaviruses. Proc Soc Exp Biol Med (In Press).

581. Luka, J., Siegert, W. and Klein, G.: Solubilization of the Epstein-Barr virus determined nuclear antigen and its characteri-zation as a DNA-binding protein. J Virol (In Press).

582. MacIntyre, R.J. and O'Brien, S.J.: Interacting gene enzyme systems in Drosophila. Annu Rev Genet 10: 281-318, 1976.

583. Mack, T.M. and Henderson, B.E.: Cancer registries for general and special uses. US-USSR Mcncgr (In Press).

584. Mack, T.M., Pike, M.C. and Casagrande, J.C.: Epidemiological methods for human risk assessment. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

585. Mack, T.M., Pike, M.C., Henderson, B.E., Pfeffer, R.I., Gerkins, V.R., Arthur, M. and Brown, S.E.: Estrogens and endometrial cancer in a retirement community. N Engl J Med (In Press).

586. Mackey, J.K., Rigden, P.M. and Green, M.: Do highly oncogenic group A human adenoviruses cause human cancer? Analysis of human tumors for adenovirus 12 transforming DNA sequences. Proc Natl Acad Sci USA 73(12): 4657-4661, 1976.

587. Maisel, J.E., Dina, D. and Duesberg, P.: Murine sarcoma viruses: The helper-independence reported for a Moloney variant is unconfirmed; distinct strains differ in the size of their RNAs. Virology 76(1): 295-312, 1977.

588. Major, E.O., Bernhardt, G., Wright, P.J. and di Mayorca, G.: Examination of the growth curve characteristic of a plaque purified human papovavirus: BK. J Virol (In Press).

589. Major, E.O., Zacharias, C., Fiori, M. and di Mayorca, G.: BK virus, a human polyomavirus, its natural history, biology and biochemistry. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

590. Marciani, D.J., Lyons, L.B. and Thompson, E.B.: Characteristics of cell membranes from somatic cell hybrids between rat hepatoma and mouse L-cells. Cancer Res 36: 2937-2944, 1976.

591. Marciani, D.J. and Okazaki, T.: Interactions of concanavalin A with chick embryo fibroblasts transformed by Rous sarcoma virus. Biochim Biophys Acta 455: 849-864, 1976.

592. Margalith, M., Gerard, G.F. and Green, M.: Studies on the DNA polymerase activity contained in particles released from human embryo cell monolayers. Biochim Biophys Acta (In Press).

593. Marks, P.A., Rifkind, R.A., Bank, A., Terada, M., Tanaka, M., Ohta, Y., Reuben, R.C. and Fibach, E.: Erythroid cell differentiation T-ansition of erythroid precursor cells to differentiated cells. Proc XVI Int Cong of Hematology, 1976, Kyoto, Japan (In Press).

594. Márquez, E.D: Binding of concanavalin A by normal, herpes virus-transformed, and trypsin-treated hamster embryo fibroblasts. Exp Cell Res 101: 425-429, 1976.

595. Márquez, E.D. and Rapp, F.: Presence of herpesvirus associated antigens on the surfaces of transformed, tumor, and metastatic cells and enhanced antigenicity of transformed cells using 5-iodo-2-deoxyuridine. Intervirology 6: 64-71, 1976.

596. Martin, D.P. and Leiseca, S.A.: A surgical procedure for cannulation of the thoracic duct of the rhesus monkey (Macaca mulatta): thoracic approach. Lab Anim Sci (In Press).

597. Martin, M.A. and Khoury, G.: Integration of DNA tumor virus genomes. Current Topics in Microbiology 73: 35-65, 1976.

598. Maruyama, K., Miller, M.F., Hixson, D.C. and Wagner, S.H.: Surface properties of mammalian C-type viruses. In Clemmesen, J. and Yohn, I.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

599. Maruyama, K., Ware, M.J., Dmochowski, L. and Johnson, D.E.: Tumor-associated antibodies in human prostatic cancer. Cancer Res (In Press).

600. Mathes, I.E., Yohn, D.S., Essex, M., Hoover, E.A., Schaller, J.P. and Olsen, R.G.: Feline oncornavirus-associated cell membrane antigen. VI. Cytotoxic antibody in cats exposed to feline leukemia virus. J Natl Cancer Inst 56: 1197-2000, 1976.

601. Mathes, L.E., John, D.S. and Olsen, R.G.: Purification of infectious feline leukemia virus from large volumes of tissue culture fluids. J Clin Microbiol 5 (In Press).

602. May, D., Miller, R.L. and Rapp, F.: The effect of phosphonoacetic acid on the in vitro replication of varicella-zoster virus. Intervirology 1977 (In Press).

603. McAllister, R.M., Gardner, M.B., Nicolson, M.O., Gilden, R.V. and Davidson, N.: RD-114 virus: Characterization and identification. In Viral Carcinogenesis: Viruses and Human Cancer. Basel, Switzerland, S. Karger (In Press).

604. McAllister, R.M., Isaacs, H., Rongey, R.W., Peer, M., Au, W. and Gardner, M.B.: Human medulloblastoma cell line. I. cytology, growth characteristics, ultrastructure and tumorigenicity. Int J Cancer 1977 (In Press).

605. McAllister, R.M. and Nicolson, M.O.: RD-114 virus: Studies of RNA of virus and DNA of provirus. Microbiology (In Press).

606. McCaffrey, R.P., Harrison, T.A., Kung, P.C., Parkman, R., Silverstone, A.E. and Baltimore, D.: Terminal deoxynuclectidyl transferase in normal and neoplastic hematopoietic cells. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Murchen, J.F. Lehmanns Verlag, 1977 (In Press).

607. McClung, H., Seth, P. and Rawls, W.E.: Relative concentrations in human sera of antibodies to cross-reacting and specific antigens of herpes simplex virus types 1 and 2. Am J Epidemiol 104(2): 192-201, 1976.

608. McClung, H., Seth, P. and Rawls, W.E.: Quantitation of antibodies to herpes simplex virus types 1 and 2 by complement-dependent antibody lysis of infected cells. Am J Epidemiol 104(2): 181-191, 1976.

609. McCoy, J.L., Jerome, L.F., Anderson, C., Cannon, G.E., Alford, T.C., Connor, R.J., Oldham, R.K. and Herberman, R.B.: Leukocyte migration inhibition by soluble extracts of MCF-7 tissue culture cell line derived from breast carcinoma. J Natl Cancer Inst 57(5): 1045-1049, 1976.

610. McDonald, R., Thakkar, B., Wolfe, L.G. and Deinhardt, F.: Characteristics of three strains of feline fibrosarcoma virus grown in cat and marmoset monkey cells. Int J Cancer (In Press).

611. McGrath, C.M., Furmanski, P., Russo, J., McCormick, J.J. and Rich, M.A.: 734B: A candidate human breast cancer virus. In Friedman, H., Crowell, R. and Prier, J. (Ed.): Tumor Virus Infection and Immunity. Baltimore, University Park Press, 1976, p 63-87.

612. McGrath, C.M., Marineau, E.J. and Voyles, B.A.: Levels of mammary tumor virus sequences in DNA of "hormone"- and "virus"-induced malignant mammary epithelial cells of the BALB/c mouse. J Virol 1977 (In Press).

613. McGrath, C.M. and Soule, H.D.: An inquiry into the involvement of non-human breast cancer viruses in the human disease: Virus expression in malignant breast ductal stem cells. In Hiatt, H.H., Watson, J.D. and Winsten, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

614. McLellan, W.L. and August, J.T.: Analysis of the envelope of Rauscher murine oncornavirus: In vitro labeling of glycopeptides. J Virol 20: 627-636, 1976.

615. Menck, H.R. and Henderson, B.E.: Occupational differences in rates of lung cancer. J of Occup Med 18: 797-801, 1976.

616. Michalides, R., Schlom, J., Pearson, J.W., Perk, K and Dahlberg, J.E.: Characterization of the oncornavirus particles in the plasma of guinea pigs with the L2C leukemia. J Virol 18: 1120-1130, 1976.

617. Michalides, R., Vlahakis, G. and Schlom, J.: A biochemical approach to the study of the transmission of mouse mammary tumor viruses in mouse strains RIII and C2H. Int J Cancer 18: 105-115, 1976.

618. Milleck, J. and Pasternak, G.: In vitro and in vivo activities of heterologous antisera against mouse leukemias. Medicina 36: 457-462, 1976.

619. Miller, R.L., Glaser, R. and Rapp, F.: Studies of an Epstein-Barr virus-induced DNA polymerase. Virology 76: 494-502, 1977.

620. Miller, R.L. and Rapp, F.: Varicella-zoster virus-induced DNA polymerase. J Gen Virol 1977 (In Press).

621. Miller, R.L. and Rapp, F.: Distinguishing cytomegalovirus, mycoplasma, and cellular DNA polymerases. J Virol 20(3): 564-569, 1976.

622. Mishra, N.K., Pant, K.J., Thomas, F.O. and Price, F.J.: Chemical-viral co-carcinogenesis: Requirement for leukemia virus expression in accelerated transformation. Int J Cancer 18: 852-858, 1976.

623. Mishra, N.K., Pant, K.J., Wilson, C.M. and Thomas, F.O.: Carcinogen-induced mutations at two separate genetic loci are not enhanced by leukemia virus infection. Nature 266: 548, 1977.

624. Moar, M. and Natarajan, A.T.: Chromosomal locations of satellite DNA in the MSWBS ascites tumour cell line. J Natl Cancer Inst (In Press).

625. Moar, M., Siegert, W. and Klein, G.: Autoradiographic detection of the EBV-associated early antigen (EA) in lymphoblastoid cell lines. In: Recent Trends in the Immunological Diagnosis of Leukemias and Lymphomas. (In Press).

626. Moar, M., Siegert, W. and Klein, G.: Detection and localization of Epstein-Barr virus-associated early antigens (EA) in single cells by autoradiography using 125 I-labelled antibodies. Intervirology (In Press).

627. Mohr, S.J. and Chirigos, M.A.: Potentiation of a tumor cell vaccine by Pyran copolymer. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 421-435.

628. Mohr, S.J. and Chirigos, M.A.: Tilorone hydrochloride as immuno-adjuvant therapy against murine neoplasias. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

629. Mohr, S.J., Chirigos, M.A., Fuhrman, F.S. and Smith, C.: Enhancement of a tumor allograft in CD2F mice by pyran copolymer. Cancer Res (In Press).

630. Molinaro, G.A., Major, E.O., Bernhardt, G., Dray, S. and di Mayorca, G.: Similar cell surface antigens on hamster cells transformed by different papovaviruses. J Immunol (In Press).

631. Montelaro, R.C., Herman, A.C. and Polognesi, D.P.: Transfer of radioiodine from iodinated oncornavirus proteins to unlabeled protein carrier during routine procedures for peptide mapping. Biochem Biophys Res Commun 75: 38-44, 1977.

632. Moore, D.H., Holben, J.A. and Charney, J.: Biological characteristics of some of the mouse mammary tumor viruses. J Natl Cancer Inst 57: 889-896, 1976.

633. Morgan, D., Ruscetti, F.W. and Gallo, R.C.: Growth of thymus derived lymphocytes from normal human bone marrow. Science 193: 1007, 1976.

634. Morrow, R.H., Kisuule, A., Pike, M.C. and Smith, P.G.: Burkitt's lymphoma in the Mengo District of Uganda: Epidemiological features and their relationship to malaria. J Natl Cancer Inst 56: 479-484, 1976.

635. Moscovici, C., Moscovici, M., Jimenez, H., Lai, M. and Vogt, P.K.: Continuous tissue culture cell lines derived from chemically induced tumors of Japanese quail. Cell 1976 (In Press).

636. Mulder, C., Fleckenstein, B. and Simonds, J.A.: Structural analysis of Herpesvirus saimiri with restriction endonucleases. Proc 9th Mtg European Tumor Virus Group, 1975, Copenhagen, Denmark (In Press).

637. Munk, K., Darai, G., Deinhardt, F., Marczynska, B., Nonoyama, M. and Silver, S.: Herpes simplex virus and neoplastic transformation. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 59-63.

638. Murasko, D.M. and Lausch, R.N.: Immunostimulation of cytomegalovirus tranformed cells and its inhibition by blocking sera. J Natl Cancer Inst 56: 1083-1085, 1976.

639. Nahmias, A. and Ashman, R.: Herpes simplex virus-associated antigens. In Herberman, R. (Ed.): Tumor Immunology. (In Press).

640. Nahmias, A. and Sawanobori, S.: The genital herpes--cervical cancer hypothesis - 10 years later. In Ito, Y. (Ed.): Viral Oncogenesis. (In Press).

641. Nardon, E., Monguet, D., Didier-Pichet, M.I. and De Thé, G.: Comparison of zymogram of three lymphoblastoid cell lines (Burkitt lymphoma) with a new microtechnique. Biomedicine (In Press).

642. Naso, R.B., Arcement, L.J., Karshin, W.L., Jamjoom, G.A. and Arlinghaus, R.B.: A fucose-deficient glycoprotein precursor to Rauscher leukemia virus gp69/71 and p12. Proc Natl Acad Sci USA 73: 2326-2330, 1976.

643. Nebert, D.W., Kouri, R.E., Yagi, H., Jerina, D.M. and Boobis, A.R.: Genetic differences in benzo(a)pyrene carcinogenic index in vivo and in mouse cytochrome P1-450 mediated benzo(a)pyrene metabolite binding to DNA in vitro. In Snyder, R. and Koscis, N. (Ed.): Reactive Intermediates. New York, Plenum Press, 1976 (In Press).

644. Nebert, D.W., Kouri, R.E., Yagi, H., Jerina, D.M. and Boobis, A.R.: Genetic differences in mouse cytochrome P1-450-mediated metabolism of benzo(a)pyrene in vitro (carcinogenic index in vivo). In Snyder, R., Jollow, D. and Gillett, J.F. (Ed.): Active Intermediates: Formation, Toxicity and Inactivation. New York, Plenum Press (In Press).

645. Nelson, K., Pollack, S.B. and Hellström, K.E.: In vitro synthesis of tumor-specific factors with blocking and antibody-dependent cellular cytotoxicity (ADC) activities. Int J Cancer (In Press).

646. Nelson, K., Pollack, S.B. and Hellström, K.E.: Specific anti-tumor responses of cultured immune spleen cells. III. Further characterization of cells which synthesize factors with blocking and antiserum dependent cellular cytotoxic (ADC) activities. Int J Cancer (In Press).

647. Nelson-Rees, W.A.: Cytogenetics as a method for cell identification. Proc Workshop on Cell Substrates for Vaccine Production, Bureau of Biologics FDA and NIAID, NIH, Bethesda, Sep 1976.

648. Nelson-Rees, W.A.: The wayward cell. In Science Year. Chicago, Ill., Field Enterprise Educational Corporation, 1977, p 310-311.

649. Nelson-Rees, W.A. and Flandermeyer, R.R.: Inter- and intra-species cellular contamination involving two new purported human breast tumor cell lines HBC and ErCa5 and other experimental cultures. Science 195: 1343-1344, 1977.

650. Nelson-Rees, W.A., Owens, R.E., Arnstein, P. and Knizzeff, A.J.: Source, alterations, characteristics and use of a new dog cell line (Cf2Th). In Vitro 12: 665-669, 1976.

651. Nepom, J.T., Hellström, I. and Hellström, K.E.: Purification and partial characterization of a tumor-specific blocking factor from sera of mice with growing chemically induced sarcomas. J Immunol 117: 1846-1852, 1976.

652. Neubauer, R.H., Rabin, H., Arnstein, P., Woodside, N.J., Valerio, M.G., Wallen, W.C., Hopkins, R.F. III and Loeb, W.F.: Characterization of a spontaneous undifferentiated carcinoma from an African green monkey (Cercopithecus aethiops). In Vitro 12: 533-539, 1976.

653. Neubauer, R.H., Wallen, W.C. and Rabin, H.: Antilymphocyte factor derived from a Herpesvirus saimiri lymphoid tumor cell line. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

654. Newgard, K.W., Cardiff, R.D. and Blair, P.B.: Human antibodies binding to the mouse mammary tumor virus: A non-specific reaction. Cancer Res (In Press).

655. Nicolson, M.O., Hariri, F., Krempin, H.M., McAllister, R.M. and Gilden, R.V.: Infectious proviral DNA in human cells infected with transformation-defective type C viruses. Virology (In Press).

656. Nilsson, K., Giovanella, B.C., Stehlin, J.S. and Klein, G.: Tumorigenicity of human hematopoietic cell lines in athymic nude mice. Int J Cancer 13: 337-344, 1977.

657. Niman, H.L., Stephenson, J.R., Gardner, M.B. and Roy-Burman, P.: Evidence for the possible etiologic involvement of endogenous type C viral gene expression in natural lymphomas of domestic cats. Nature (In Press).

658. Niman, H.L., Stephenson, J.R., Gardner, M.B. and Roy-Burman, P.: RD-114 and FeLV genome expression in natural lymphomas of domestic cats. Nature 266: 357-360, 1977.

659. Nishioka, K., Levin, A.G. and Simons, M.J.: Report of WHO-assisted workshop on hepatitis B antigen. Bull WHO (In Press).

660. Nkrumah, F., Henle, W., Henle, G., Herberman, R., Perkins, V. and Depue, R.: Burkitt's lymphoma: Its clinical course in relation immunologic reactivities to Epstein-Barr virus and tumor related antigens. J Natl Cancer Inst 57: 1051-1056, 1976.

661. Nomura, S. and Fischinger, P.J.: Rapid screening assay for revertants of MSV-transformed cells. In Prescott, D.M. (Ed.): Methods in Cell Biology. New York, Academic Press, Inc., 1976, Vol 14, p 229-236.

662. Nomura, S., Fischinger, P.J., Hartley, J.W. and Mattern, C.F.T.: Induction of a novel transforming virus (MSV-Z) from sarcoma positive leukemia negative 3T3FI mouse cells. Virology 69: (In Press).

663. Nonoyama, M., Tanaka, A. and Glaser, R.: Expression of latent Epstein-Barr virus genomes in human epithelial/Burkitt hybrid cells. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

664. Nonoyama, M., Tanaka, A. and Yajima, Y.: Replication and latency of Epstein-Barr virus. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 291-296.

665. Noonan, K.D., Bouck, N. and di Mayorca, G.: The dissociation of the surface architecture described by enhanced lectin agglutinability and the transformed phenotype expressed as growth in suspension. J Cell Physiol (In Press).

666. Nooter, K., Bentvelzen, P., Zurcher, C. and Rhim, J.S.: Detection of human C-type 'helper' viruses in human leukemic bone marrow with murine sarcoma virus-transformed human rat nonproducer cells. Int J Cancer 19: 59-65, 1977.

667. Nowinski, R.C. and Baron, J.: Genetic and immunological controls of murine leukemia viruses in AKR mice. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

668. Nowinski, R.C. and Doyle, T.: Antibody to murine leukemia virus: Genetic control by the Ir-1 locus in PL mice. J Immunol 117: 350-351, 1976.

669. Nowinski, R.C. and Doyle, T.: Decreased immunity to viral antigens and increased expression of endogenous leukemia viruses in athymic (nude) mice. J Virol (In Press).

670. Nowinski, R.C., Hays, E.F., Doyle, T., Linkhart, S., Medeiros, E. and Pickering, R.: Defective oncornaviruses produced by murine leukemia cells in cluture. Virology (In Press).

671. Nowinski, R.C. and Miller, E.C.: Endogenous oncornaviruses in chemically-induced transformation. II. Effect of virus production in vivo. J Natl Cancer Inst 57: 1347-1350, 1976.

672. Nowinski, R.C. and Watson, A.: Immune response of the mouse to the major core protein (p30) of ecotropic leukemia viruses. J Immunol 117: 693-696, 1976.

673. Nudel, U., Ramierez, F., Marks, P.A. and Bank, A.: Preparative polyacrylamide gel electrophoretic purification of human alpha and beta globin messenger RNA's. J Biol Chem 1977 (In Press).

674. Nudel, U., Salmon, J.E., Terada, M., Bank, A., Rifkind, R.A. and Marks, P.A.: Differential effects of chemical inducers on the expression of a beta globin genes in murine erythroleukemia cells. Proc Natl Acad Sci USA 1977 (In Press).

675. Oakes, J.E., Hyman, R.W. and Rapp, F.: Genome location of poly-adenylated transcripts of herpes simplex virus type 1 and type 2 DNA. Virology 75: 145-154, 1976.

676. O'Brien, S.J.: 1977 Editor, Isozyme Bulletin, Vol 10, 1977, p 1-90. National Cancer Institute, Bethesda, Md.

677. O'Brien, S.J.: Bvr-1: A C-type RNA virus restriction locus in the feline cellular genome: Identification, location and phenotypic characterization in cat x mouse somatic cell hybrids. Proc Natl Acad Sci USA 73: 4618-4622, 1976.

678. O'Brien, S.J.: Isozymes and allozymes as genetic foot-prints of cultured cells. In Petricianna, J. (Ed.): Proc of the FDA Conf on Cell Substrates for Vaccine Production, 1976, p 46-59.

679. O'Brien, S.J. and Boone, C.W.: Expression of feline leukemia virus antigens on cat lymphoma cells: Kinetics of biosynthesis. J Gen Virol (In Press).

680. O'Brien, S.J., Boone, C.W., Simonson, J.M. and Austin, P.: The paired label assay for cell surface antigens. Tissue Culture Assoc Manual 2: 423-427, 1976.

681. O'Brien, S.J., Kleiner, G., Olson, R. and Shannon, J.: Enzyme polymorphisms as genetic signatures in human cell cultures. Science 195: 1345-1348, 1977.

682. O'Brien, S.J. and MacIntyre, F.J.: Biochemistry and genetics of enzymes and specific proteirs in Drosophila melanogaster. In Wright, T.R.F. and Ashburner, M. (Ed.): Genetics and Biology of Drosophila. New York, Academic Press, Inc., 1976, Vol II (In Press).

683. O'Brien, S.J., Simonson, J.M. and Boone, C.W.: Expression of viral structural proteins on murine cell surface in association with the production of murine leukemia virus. J Gen Virol 33: 355-360, 1976.

684. Ogden, J.D., Wolfe, L.G. and Deinhardt, F.: Breeding saguinus and callithrix species of marmosets under laboratory conditions. Primates in Medicine. Basel, S. Karger AG (In Press).

685. Ogino, T. and Rapp, F.: Deoxycytidine kinase activity from rabbit kidney cells infected with herpes simplex virus type 1 or 2. Proc Soc Exp Biol Med (In Press).

686. Ohno, S., Luka, J., Lindahl, T. and Klein, G.: Identification of a purified complement fixing antigen as the Epstein-Barr virus-determined nuclear antigen (EBNA) by its binding to metaphase chromosomes. Proc Natl Acad Sci USA (In Press).

687. Ohno, T. and Spiegelman, S.: Antigenic relatedness of the DNA polymerase of human breast cancer particles to the enzyme of the Mason-Pfizer monkey virus. Proc Natl Acad Sci USA (In Press).

688. Ohno, T., Sweet, R.W., Hu, R., DeJak, D. and Spiegelman, S.: Purification and characterization of the DNA polymerase of human breast cancer particles. Proc Natl Acad Sci USA 74: 764-768, 1977.

689. Ohta, Y., Tanaka, M., Terada, M., Miller, O.J., Bank, A., Marks, P.A. and Rifkind, R.A.: Erythroid cell differentiation murine erythroleukemia cell variant with a unique pattern of induction by polar compounds. Proc Natl Acad Sci USA 73(4): 1232-1236, 1976.

690. Ohtsuki, Y., Seman, G., Bowen, J.M., Scanlon, M.D. and Dmochowski, L.: Detection of viral antigens in mouse and rat tumor cells by immunoelectron microscopy following periodate-lysine-paraformaldehyde (PLP) fixation. In Bailey, G.W. (Ed.): 34th Annu Proc Electron Microscopy Soc Am. Miami Beach, Fla., 1976, p 252.

691. Okabe, H., Gilden, R.V., Hatanaka, M., Stephenson, J.R., Tronick, S.R., Gallagher, R.E., Gallo, R.C. and Aaronson, S.A.: Immunologic and biochemical characterization of type C virus isolates from cultured human AML cells. Nature (In Press).

692. Okabe, H., Twiddy, R., Gilden, R.V., Hatanaka, M., Hoover, E.A. and Olsen, R.G.: FeLV related sequences in DNA from a FeLV-free cat colony. Virology 69: 798-801, 1976.

693. Okayama, M., Yoshimura, M., Muto, M., Chi, J., Roth, S.L. and Kaji, A.: Transformation of chicken chondrocytes by Rous sarcoma virus. Cancer Res 37: 712-717, 1977.

694. Oldstone, M.B.A., Del Villano, B.C. and Dixon, F.J.: Autologous immune responses to the major oncornavirus polypeptides in unmanipulated AKR/J mice. J Virol 18(1): 176-181, 1976.

695. Oldstone, M.B.A., Lampert, P.W., Lee, S. and Dixon, F.J.: Pathogenesis of the slow disease of the central nervous system associated with WM 1504E virus. I. Relationship of strain susceptibility and viral replication to disease. Am J Pathol (In Press).

696. Oleske, J.M., Ashman, R., Kohl, S., Shore, S., Wood, F.A. and Nahmias, A.: Polymorphonuclear leukocytes ad mediators of antibody-dependent cellular cytotoxicity to herpes simplex virus-infected target cells. Clin Exp Immunol 27: 446-453, 1977.

697. Olsen, R.G., Hoover, E.A., Mathes, L.E., Heding, L. and Schaller, J.P.: Immunization against feline oncornavirus disease using a killed tumor cell vaccine. Cancer Res 36: 3642-3649, 1976.

698. Olsen, R.G., Hoover, E.A., Schaller, J.P., Mathes, L.E. and Wolff, L.H.: Abrogation of resistance to feline oncornavirus disease by immunizing with killed feline leukemia virus. Cancer Res (In Press).

699. Olshevsky, U. and Becker, Y.: Synthesis and transport of herpes simplex virus proteins in arginine-deprived BSC-1 cells. Isr J Med Sci 12: 1298-1307, 1976.

700. Olweny, C.L.M., Atine, I., Kaddu-Mukasa, A., Owor, R., Klein, G., Lindahl, T., Andersson, M., Zech, L. and Henle, W.: Epstein-Barr virus genome studies in Burkitt and non-Burkitt lymphomas in Uganada. J Natl Cancer Inst (In Press).

701. Oskarsson, M.K., Long, C., Robey, W.G., Scherer, M.A., Vande Woude, G.F.: Phosphorylation and nucleic acid binding properties of M1 Moloney murine sarcoma virus-specific P60 gag. J Virol 1977 (In Press).

702. Oskarsson, M.K., Scherer, M.A., Robey, W.G., Long, C. and Vande Woude, G.F.: Phosphorylation of Moloney murine sarcoma virus specific P60 and its DNA and RNA binding properties. In Clark, R.E. and Cumley, R.W. (Ed.): Yearbook of Cancer. Chicago, Yearbook Medical Publishers, p 504-506.

703. Osugi, Y., Suzuki, S. and Takagaki, Y.: Antitumor and immuno-suppressive effects of mycophenolic acid derivatives. Cancer Res 36: 2923-2927, 1976.

704. Otten, J.A., Quarles, J.M. and Tennant, R.W.: Cell division requirement for activation of murine leukemia virus in cell culture by irradiation. Virology (In Press).

705. Overturf, G.D., Powars, D.P., McAllister, R.M., Pegelow, C. and Charman, H.P.: Acute lymphocytic leukemia following Reye's syndrome treated by multiple exchange transfusions. Med Ped Oncology (In Press).

706. Owens, R.B.: Selective culture of mammalian epithelial cells. In Prescott, D.M. (Ed.): Methods in Cell Biology. New York, Academic Press, Inc., Vol XIV, 1976.

707. Owens, R.B., Smith, H.S., Nelson-Rees, W.A. and Springer, E.L.: Selective cultivation of human epithelial cells. J Natl Cancer Inst (In Press).

708. Owens, R.B., Smith, H.S., Nelson-Rees, W.A. and Springer, E.L.: Epithelial cell cultures from normal and cancerous human tissues. J Natl Cancer Inst 56: 843-849, 1976.

709. Pachmann, K. and Leibold, W.: Insolubilization of protein antigens on polyacrylic plastic beads using poly-L-lysine. J. Immunol Methods 12: 81-89, 1976.

710. Padmanabhan, R., Padmanabhan, R. and Green, M.: Evidence for palindromic sequences near the termini of adenovirus 2 DNA. Biochem Biophys Res Commun (In Press).

711. Pagano, J.S.: Molecular biological studies implicating the Epstein-Barr virus in the etiology of Burkitt's lymphoma and certain other cancers. In Gallo, R. (Ed.): Cancer Research: Cell Biology, Molecular Biology and Tumor Virology. Cleveland, Ohio, CRC Press, Inc., 1977 (In Press).

712. Pagano, J.S., Huang, C.-H. and Huang, Y.-T.: The Epstein-Barr virus genome in infectious mononucleosis. Nature 263: 787-789, 1976.

713. Panasenko, S.M., Cameron, J.R., Davis, R.W. and Lehman, I.R.: Five hundred-fold overproduction of E. coli DNA ligase after induction of a lysogen containing hybrid lambda DNA constructed in vitro. Science 196: 188-189, 1977.

714. Papadopoulos, N.M.: Lactate dehydrogenase isoenzymes clinical applications. Isozyme Bulletin (In Press).

715. Papamatheakis, J.D., Chirigos, M.A. and Schultz, R.M.: Effect of dose, route and timing of Pyran copolymer therapy against the Madison lung carcinoma. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

716. Papas, T.S., Chirikjian, J.G., Woods, W.A. and Chirigos, M.A.: Active side probes and the selective inhibition of RNA tumor viral DNA polymerases. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 369-375.

717. Papas, T.S., Marciani, D.J., Samuel, K. and Chirikjian, J.G.: Mechanism of release of active alpha subunit from dimeric alpha beta avian myeloblastosis virus DNA polymerase. J Virol (In Press).

718. Papas, T.S., Pry, T.W. and Marciani, D.J.: Inactivation of avian myeloblastosis virus DNA polymerase by specific binding of pyridoxal 5'-phosphate to deoxynucleoside triphosphate binding site. J Biol Chem 252: 1425-1430, 1977.

719. Papas, T.S., Pry, T.W., Schafer, M.A. and Sonstegard, R.A.: Presence of DNA polymerase in lymphosarcoma in northern pike (Esox lucius). Cancer Res (In Press).

720. Papas, T.S., Reuzi, G.R. and Martin, J.W.: Immunological distinction between ribonucleose H activity of alpha and alpha-beta forms of avian myeloblastosis virus (AMV) reverse transcriptase. Virology (In Press).

721. Papas, T.S. and Schafer, M.A.: The effect and mode of action of anthracycline compounds on oncornavirus DNA polymerase activity. Ann NY Acad Sci (In Press).

722. Papas, T.S. and Schafer, M.A.: The inhibition of Rauscher leukemia virus of avian myeloblastosis virus DNA polymerase by anthracycline compounds. Ann NY Acad Sci 284: 566-575, 1977.

723. Paranjpe, M.S., Boone, C.W. and Takeichi, N.: Specific paralysis of the anti-tumor cellular immune response produced by growing tumors studied with a radioisotopic footpad assay. Ann NY Acad Sci 276: 254-259, 1976.

724. Parker, J.C., Reynolds, R.K., Igel, H.J., Rowe, W.P. and Lewis, A.M., Jr.: Lymphocytic choriomeningitis infection in the fetal, neonatal, and adult Syrian hamster (Mesocricetus auratus). Infection & Immunity (In Press).

725. Parks, W.P., Hubbell, E.S., Goldberg, R.J., O'Neill, F.J. and Scolnick, E.M.: High frequency variation in mammary tumor virus expression in cell culture. Cell (In Press).

726. Parks, W.P. and Scolnick, E.M.: In vitro translation of Harvey murine sarcoma viral ribonucleic acid. J Virol (In Press).

727. Pasternak, L., Jenssen, H.I., Köhler, H. and Pasternak, G.: Cross-reactions among mouse tumors of different etiology as detected by macrophage electrophoretic mobility (MEM) test. Eur J Cancer 12: 389-393, 1976.

728. Pater, M., Hyman, R.W. and Rapp, F.: Isolation of herpes simplex virus DNA from the "Hirt supernatant." Virology 75: 481-483, 1976.

729. Paul, P.S., Pomeroy, K.A., Sarma, P.S., Johnson, K.E., Barnes, D. M., Kumar, M.C. and Pomercy, B.S.: Naturally occurring reticuloendotheliosis in turkeys: Transmission. J Natl Cancer Inst (In Press).

730. Pearson, G.R., Coates, H., Neel, B., Levine, P.H., Ablashi, D.V. and Easton, J.M.: Clinical evaluation of EBV serology in American NPC patients. 2nd Int Symp on Etiology and Control of Nasopharyngeal Carcinoma, Kyoto, Japan, Apr 1977 (In Press).

731. Peebles, P.T., Gerwin, B.I. and Scolnick, E.M.: Murine sarcoma virus defectiveness: Serological detection of only helper virus reverse transcriptase in sarcoma virus rescued from nonmurine S+Lcells. Virology (In Press).

732. Peebles, P.T., Scolnick, E.M. and Howk, R.S.: Increased sarcoma virus RNA in cells transformed by leukemia viruses: Model for leukemogenesis. Science 192: 1143-1145, 1977.

733. Périés, J. and Todaro, G.J.: A hemagglutination assay for the primate syncytium-forming (foamy) viruses. J Gen Virol 34: 195-199, 1977.

734. Perk, K. and Pearson, J.W.: Therapeutic trial of an animal analog for meingeal leukemia with MeCCNU. Isr J Med Sci (In Press).

735. Perlin, E., Levine, P.H., McCoy, J.L., Dean, J.H. and Herberman, R.: Hodgkin's disease in siblings: A family study. Oncology 33: 116-118, 1976.

736. Peters, G., Harada, F., Dahlberg, J.E., Panet, A., Haseltine, W.A. and Baltimore, D.: The low molecular weight RNAs of Moloney murine leukemia virus: Identification of the primer for RNA-directed DNA synthesis. J Virol 21: 1031-1041, 1977.

737. Peters, R.L., Sass, B., Stephenson, J.R., Al-Ghazzouli, I.K., Hino, S., Donahoe, R.M., Kende, M., Aaronson, S.A. and Kelloff, G.J.: Immunoprevention of X-ray-induced leukemias in the C57BL mouse. Proc Natl Acad Sci USA (In Press).

738. Peto, R. and Pike, M.C.: The analysis of clinical trials in cancer. Br J Cancer (In Press).

739. Petrányi, G., Kiessling, R., Povey, S., Klein, G., Herzenberg, L. and Wigzell, H.: The genetic control of natural killer cell activity and its association with in vivo resistance against a Moloney lymphoma isograft. Immunogenetics 3: 15-28, 1976.

740. Phillips, L.A. and Pang, R.H.L.: The detection and characterization of polynucleotide sequences in the RNA of mammalian oncornaviruses. In Clemmesen, J. and Yohr, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 460-464.

741. Phillips, L.A. and Pang, R.H.L.: Poly(C)- and Poly(G)-rich tracts in the RNA of oncornaviruses. In Nieburgs, H.E. (Ed.): 3rd Int Symp on Detection and Prevention of Cancer. New York, Marcel Dekker, Inc., Apr 1976 (In Press).

742. Phillips, S.M., Stephenson, J.R. and Aaronson, S.A.: Genetic factors influencing mouse type C RNA virus induction by naturallyoccurring B-cell mitogens. J Immunol 118: 662-666, 1977.

743. Phillips, S.M., Stephenson, J.R., Greenberger, J.S., Lane, P.E. and Aaronson, S.A.: Induction of a type C RNA virus by lipopoly-saccharide: Activity in lipid-A portion upon T and B lymphocytes. J Immunol 116: 1123-1128, 1976.

744. Pickett, R.A., Geltosky, J.E. and Lerner, R.A.: Developmentally regulated phosphorylation of plasma membrane proteins in Dictyostelium discoideum. Cell (In Press).

745. Pike, M.C., Edmondson, H.A., Benton, B. and Henderson, B.E.: Liver adenomas and oral contraceptives. Conf on Cell Proliferation, RNA Tumor Virus Mtg, May 1977, Cold Spring Harbor Laboratory, New York, Vol 4 (In Press).

746. Pike, M.C. and Henderson, B.E.: "Clustering" of cancer cases. US-USSR Monogr (In Press).

747. Pike, M.C. and Smith, P.G.: A note on detecting household aggregation of disease. Biometrics (In Press).

748. Pitha, P.M., Rowe, W.P. and Cxman, M.N.: Effect of interferon on exogenous, endogenous, and chronic murine leukemia virus infection. Virology (In Press).

749. Pitha, P.M., Staal, S.P., Bolognesi, D.P., Denny, T.P. and Rowe, W.P.: Effect of interferon on murine leukemia virus infection. II. Effect on the synthesis of viral components in exogenous infection. Virology (In Press).

750. Powell, K.L., Mirkovic, R. and Courtney, R.J.: Comparative analysis of polypeptides induced by type 1 and type 2 strains of herpes simplex virus. Intervirology 8: 18-29, 1977.

751. Powell, K.L. and Purifoy, D.J.M.: DNA-binding proteins of cells infected by herpes simplex virus type 1 and type 2. Intervirology 7: 225-239, 1976.

752. Prather, S.O. and Lausch, R.N.: Kinetics of serum factors mediating, blocking, unblocking and antibody-dependent cellular cytotoxicity in hamsters given isografts of PARA-7 tumor cells. Int J Cancer (In Press).

753. Prather, S.O. and Rapp, F.: Distinguishing cytomegalovirus, mycoplasma, and cellular DNA polymerases. J Virol 20: 564-569, 1976.

754. Premkumar-Reddy, E., Devare, S.G., Vasudev, R. and Sarma, P.S.: A simplified radioimmunoassay for viral antigens: Use of staphylococcus aureus as an adsorbant for antigen-antibody complexes. J Natl Cancer Inst (In Press).

755. Premkumar-Reddy, E., Price, P.J., Chung, K.C. and Sarma, P.S.: Continuous culturing of murine splenic B-lymphocytes: Synthesis and surface deposition of IgM and putative IgD molecules. Cell 8: 397-403, 1976.

756. Prevost, J.-M., Pearson, G.R., Wallen, W.C., Rabin, E. and Qualtiere, L.F.: Antibody responses to membrane antigens in monkeys infected with herpesvirus saimiri. Int J Cancer 18: 679-686, 1976.

757. Price, P.J., Auletta, A.E., King, M.P., Hugunin, P.M. and Huebner, R.J.: The co-carcinogenic activity of 4-nitropyridine-1-oxide (4-NPO) and prevention of transformation by type-specific anti-viral antibodies. In Vitro 12: 595-598, 1976.

758. Price, P.J., Suk, W.A., Peters, R.L., Gilden, R.V. and Huebner, R.J.: Chemical transformation of rat cells infected with xenotropic type C RNA virus and its suppression by virus-specific antiserum. Proc Natl Acad Sci USA 74: 579-581, 1977.

759. Price, P.J., Suk, W.A., Skeer, P.A., Chirigos, M.A., Spahn, G.J. and Huebner, R.J.: Geldanamycin inhibition of 3-methylcholanthrene induced rat embryo cell transformation. Proc Soc Exp Biol Med (In Press).

760. Priori, E.S., Ilyin, K.V., Dmochowski, L. and Fine, I.L.: Immunological relationship between an oncornavirus isolate from HEp-2 cells, Mason-Pfizer monkey virus, and human tumor cells. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 488-490.

761. Proffitt, M.R., Hirsch, M.S. and Black, P.H.: Viruses, autoimmunity and murine lymphoma. In Talal, N. (Ed.): Autoimmunity. New York, Academic Press (In Press).

762. Proffitt, M.R., Hirsch, M.S., Ellis, D.A., Gheridian, B. and Black, P.H.: Immunological mechanisms in the pathogenesis of virus-induced murine leukemia. III. Target cell specificity of autoreactive thymocytes. J Immunol 117: 11-15, 1976.

763. Purifoy, D.J.M. and Powell, K.L.: Interference between strains of type 1 and type 2 herpes simplex virus. Virology (In Press).

764. Putnam, D.L. and Rhim, J.S.: Characterization of bromodeoxyuridine-induced guinea pig type B retravirus. Fed Proc 1977 (In Press).

765. Quarles, J.M., Sega, M.W., Schenley, C.K. and Tennant, R.W.: Rapid screening for chemical carcinogens: Transforming activity of selected nitroso compounds detected in a transplacental host-mediated culture system. Natl Cancer Inst Monogr (In Press).

766. Rabin, H.: Virus-associated hematopoietic neoplasms of nonhuman primates. Advances in Medical Primatology (In Press).

767. Rabin, H., Neubauer, R.H., Woodside, N.J., Cicmanec, J.L., Wallen, W.C., Lapin, B.A., Agrba, V., Yakoleva, L. and Chuvirov, G.N.: Virological studies of baboon (Papio hamadryas) lymphoma: Isolation and characterization of foamyviruses. J Med Primatol (In Press).

768. Rabin, H., Pearson, G.R., Wallen, W.C., Neubauer, R.E., Cicmanec, J.L. and Levy, B.: Comparative studies with different strains of Epstein-Barr virus in owl monkeys and marmosets. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

769. Rand, K.H., Reed, B., Broseus, I., Hampar, B., Gilder, R.V. and Long, C.: Mixed culture cytopathology between oncornavirus indicator lines, K.C. and XC, and 'virus-free' human choriocarcinoma cells. J Natl Cancer Inst (In Press).

770. Rangan, S.R.S., Martin, L.N., Enright, F.M. and Allen, W.P.: Herpesvirus saimiri-induced malignant lymphoma in rabbits. J Natl Cancer Inst (In Press).

771. Rapaport, E. and Zamecnik, P.C.: Incorporation of adenosine into ATP: Formation of compartmentalized ATP. Proc Natl Acad Sci USA 73: 3122-3125, 1976.

772. Rapaport, E. and Zamecnik, P.C.: The presence of diadenosine 5',5'''-p1,p4-tetraphosphate (Ap4A) in mammalian cells in levels varying widely with the proliferative activity of the tissue: A possible positive "pleiotypic activator." Proc Natl Acad Sci USA 73: 3984-3988, 1976.

773. Rapp, F.: Transformation in vitro by DNA tumor viruses. In Gallo, R.C. (Ed.): Recent Advances in Cancer Research. Cleveland, Ohio, CRC Press, Inc., 1976 (In Press).

774. Rapp, F.: Viruses as an etiological factor in cancer. In Yarbro, J.W. (Ed.): Seminars in Oncology. New York, Grune & Stratton (In Press).

775. Rapp, F.: New properties of mammalian cells transformed by herpes simplex and cytomegaloviruses. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977, p 461-470.

776. Rapp, F. and Buss, E.R.: Comparison of herpes simplex virus isolates using a quantitative selection assay for transformation. Intervirology 6: 72-82, 1976.

777. Rapp, F. and Hutt, R.: In vitro transformation of mammalian cells by human herpesviruses. In Borek, C. and King, D.W. (Ed.): Advances in Pathobiology, Herpes Viruses. New York, Stratton Intercontinental Medical Book Corp. (In Press).

778. Rapp, F., Iltis, J., Oakes, J.E. and Hyman, R.W.: A novel approach to study the DNA of herpes zoster virus. Intervirology 1977 (In Press).

779. Rapp, F. and Kemeny, B.A.: Oncogenic potential of herpes simplex virus in mammalian cells following photodynamic inactivation. Photochem Photobiol 25: 335-337, 1977.

780. Rapp, F. and Li, J.H.: Transforming activity of viruses after dye-light inactivation. In Regan, J.D. (Ed.): Photomedicine: Light in Disease and Therapy. New York, Raven Press (In Press).

781. Rapp, F. and Reed, C.L.: The viral etiology of cancer: A realistic approach. Cancer 1977 (In Press).

782. Rapp, F. and Westmoreland, D.: Cell transformation by DNA-containing viruses. Biochim Biophys Acta 458: 167-211, 1976.

783. Rasheed, S., Freeman, A.E., Gardner, M.B. and Huebner, R.J.: Acceleration of transformation of rat embryo cells by rat type C viruses. J Virol (In Press).

784. Rasheed, S., Gardner, M.B. and Chan, E.: Amphotropic host range of naturally occurring wild mouse leukemia virus. J Virol 19: 13-18, 1976.

785. Rasheed, S., Gardner, M.B., Rongey, R.W., Nelson-Rees, W.A. and Arnstein, P.: Human bladder carcinoma: Characterization of two new tumor cell lines and search for tumor viruses. J Natl Cancer Inst 58: 881-890, 1977.

786. Rasheed, S., Toth, E. and Gardner, M.B.: Characterization of purely ecotropic and amphotropic naturally occurring wild mouse leukemia viruses. Intervirology (In Press).

787. Rawls, W.E. and Adam, E.: Herpes simplex viruses and human malignancies. In Hiatt, H.E., Watson, J.D. and Winston, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

788. Ray, U., Soeiro, R. and Fields, B.N.: Viral envelope synthesis during restricted infection of Friend leukemia virus. J Virol (In Press).

789. Reed, C.L. and Rapp, F.: Induction of murine p30 by superinfecting herpesviruses. J Virol 19: 1028-1033, 1976.

790. Rein, A., Kashmiri, S.V.S., Bassin, R.H., Gerwin, B.I. and Duran-Troise, G.: Phenotypic mixing between N- and B-tropic murine leukemia viruses. In Baltimore, D., Huang, A.S. and Fox, C.E. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976. (In Press).

791. Rein, A., Kashmiri, S.V.S., Bassin, R.H., Gerwin, B.I. and Duran-Troise, G.: Studies on the mechanism of Fv-1 restriction: Properties of determinants specifying viral tropism. In Baltimore, D., Huang, A.S. and Fox, C.E. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976, p 269-282.

792. Reitz, M.S., Miller, N.R., Wong-Staal, F., Gallagher, R.E., Gallo, R.C. and Gillespie, D.H.: Primate type C virus nucleic acid sequences (woolly monkey and baboon types) in tissues from a patient with acute myelogerous leukemia and in viruses isolated from cultured cells of the same patient (cytoplasmic virus-like particles/molecular hybridization/RNA tumor viruses/ human type C viruses). Proc Natl Acad Sci USA (In Press).

793. Reuben, R.C., Wife, R.L., Breslow, R., Rifkind, R.A. and Marks, P.A.: A new group of potent inducers of differentiation in murine erythroleukemia cells. Proc Natl Acad Sci USA 73(3): 862-866, 1976.

794. Reynolds, F.H., Hanson, C.A., Aaronson, S.A. and Stephenson, J.R.: Type C viral gag gene expression in chicken embryo fibroblasts and avian sarcoma virus transformed mammalian cells. J Virol (In Press).

795. Rhim, J.S.: Transformation of human cells in culture by chemical carcinogens. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

796. Rhim, J.S., Cho, H.Y., Huebner, R.J. and Gilden, R.V.: Characterization of Kirsten sarcoma virus transformation of human cells and isolation of nonproducer human cells. In Clemmesen, J. and Yohn, I.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 84-87.

797. Rhim, J.S., Cho, H.Y., Kim, J.M. and Green, I.: Induction and characterization of virus-free guinea pig tumors induced by Kirsten sarcoma virus. J Natl Cancer Inst (In Press).

798. Rhim, J.S., Putnam, D.L., Arnstein, P., Huebner, R.J. and McAllister, R.M.: Characterization of human cells transformed in vitro by N-methyl-N'-nitro-N-nitrosoguanidine. Int J Cancer (In Press).

799. Rhim, J.S., Vernon, M.L. and Green, I.: In vitro studies on I2C leukemia: Isolation of guinea pig herpes virus from mink cells after cocultivation with leukemic cells. Fed Proc 1977 (In Press).

800. Rich, M.A., Furmanski, P., McGrath, C.M., McCormick, J.J., Russo, J. and Soule, H.D.: The eitology of breast cancer. In Menon, K.M.J. and Reed, J.R. (Ed.): Steroid Hormone Action and Cancer. New York, Plenum Press, 1976, p 15-27.

801. Rie, E.J., Rie, I.P. and Buehring, G.C.: Normal short-term baboon mammary epithelial cell line. J Natl Cancer Inst 57: 607-611, 1976.

802. Rieche, K., Arndt, A. and Pasternak, G.: Cellular immunity in mammary cancer patients as measured by the leukocyte migration test (LMT). A follow-up study. Int J Cancer 17: 212-218, 1976.

803. Rinaldo, C.R., Jr., Hirsch, M.S. and Black, P.H.: Activation of latent viruses following bone marrow transplantation. Transplant Proc 8: 669-672, 1976.

804. Ringold, G.M., Blair, P.B., Bishop, J.M. and Varmus, H.E.: Nucleotide sequence homologies among mouse mammary tumor viruses. Virology (In Press).

805. Ringold, G.M., Cardiff, R.D., Varmus, H.E. and Yamamoto, K.R.: Infection of cultured rat hepatoma cells by mouse mammary tumor virus. Cell 10: p 11-18, 1977.

806. Ritzi, E., Baldi, A. and Spiegelman, S.: The purification of a gs antigen of the murine mammary tumor virus and its quantitation by radioimmunoassay. Virology 75: 188-197, 1976.

807. Ritzi, E., Martin, D.S., Stolfi, R.L. and Spiegelman, S.: Plasma levels of a viral protein as a diagnostic signal for the presence of mammary tumor: The effect of tumor removal. J Exp Med 145: 999-1013, 1977.

808. Ritzi, E., Martin, D.S., Stolfi, R.L. and Spiegelman, S.: Plasma levels of a viral protein as a diagnostic signal for the presence of tumor: The murine mammary tumor model. Proc Natl Acad Sci USA 73(11): 4190-4194, 1976.

809. Ritzi, E.M. and Stylos, W.A.: Prostaglandin production in cultures of BALB/3T3 and SV3T3 mouse fibroblasts. J Natl Cancer Inst 56: 529-533, 1976.

810. Robbins, K.C., Cabradilla, C., Stephenson, J.R. and Aaronson, S.A.: Origin of a B-tropic leukemia virus of the BALB/c mouse strain. Proc Natl Acad Sci USA (In Press).

811. Robert-Guroff, M., Schrecker, A.W., Brinkman, B.J. and Gallo, R.C.: DNA polymerase gamma of human lymphoblasts. Biochemistry 1977 (In Press).

812. Robey, W.G., Graham, B.J., Harris, C.L., Madden, M.J., Pearson, G.F. and Vande Woude, G.F.: Persistent herpes simplex virus infections established in two Burkitt lymphoma derived cell lines. J Gen Virol 32: 51-62, 1976.

813. Robey, W.G., Oskarsson, M.K., Vande Woude, G.F., Nash, R.B., Arlinghaus, R.B., Haapala, D.K. and Fischinger, P.J.: Cells transformed by certain strains of Moloney sarcoma virus contain murine p60. Cell 10: 79-89, 1977.

814. Roboz, J.P., Bekesi, J.G. and Holland, J.F.: Effectiveness of exogenous mouse interferon in AKR leukemia. In Chirigos, M.A. (Ed.): Control of Neoplasia by Modulation of the Immune System. New York, Raven Press, 1977, p 369.

815. Roboz, J.P., Suzuki, R., Hunt, R.D. and Bekesi, J.G.: Preclinical toxicological study of phosphonoacetic acid: Determination in blood by selected ion monitoring. Biomed Mass Spectrum 4(3): 1977.

816. Rosén, A., Britton, S., Gergely, P., Jondal, M. and Klein, G.: Polyclonal Ig production follows EB-virus infection of human lymphocytes in vitro. Nature (In Press).

817. Rosen, J.M.: Isolation and characterization of purified rat casein messenger RNA. Biochemistry 15: 5263, 1976.

818. Rosen, J.M. and Barker, S.W.: Quantitation of casein messenger RNA sequences using a specific complementary DNA hybridization probe. Biochemistry 15: 5272, 1976.

819. Roth, S.I., Delotto, R. and Kaji, A.: Changes in leucine aminotransferase isozymes by viral transformation and its correlation with the isozyme changes occurring during differentiation. Cancer Res 37: 1977.

820. Rothenberg, E. and Baltimore, D.: Increased length of DNA made by virions of murine leukemia virus at limiting magnesium ion concentration. J Virol 21: 168-178, 1977.

821. Roy-Burman, P., Dougherty, M., Pal, B.K., Charman, H.P., Klement, V. and Gardner, M.B.: Assay for type C virus in mouse sera based on particulate reverse transcriptase activity. J Virol 19: 1107-1110, 1976.

822. Rundell, A.K., Tegtmeyer, P., Wright, P.J. and di Mayorca, G.: Comparison of SV40 and BKV protein A. Virology (In Press).

823. Ruscetti, S.K., Scolnick, E.M. and Parks, W.P.: Natural immunity in cats to feline leukemia viral antigens. J Immunol 117: 2029-2035, 1976.

824. Russo, J., Bradley, R., McGrath, C.M. and Russo, I.E.: Collagen coated cellulose sponge culture of a human breast carcinoma cell line, (MCF-7): Scanning and transmission electron microscopic study. Cancer Res 1977 (In Press).

825. Russo, J., McGrath, C.M. and Russo, I.H.: An experimental animal model for the study of human scirrhous carcinoma. J Natl Cancer Inst 57: 1253-1259, 1976.

826. Russo, J., McGrath, C.M., Russo, I.H. and Rich, M.A.: Tumoral growth of a human breast cancer cell line (MCF-7) in athymic mice. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

827. Russo, J., Russo, I.H. and Wells, P.: Histochemical markers for identifying cellular types in mouse mammary carcinoma. Fed Proc (In Press).

828. Russo, J., Saby, J., Isenberg, W. and Russo, I.H.: Pathogenesis of mammary carcinoma induced in rats by 7-12 (DMBA). J Natl Cancer Inst 59: 1020-1035, 1977.

829. Russo, J. and Wells, P.: Ultrastructural localizations of adenosine triphosphatase activity in resting mammary gland. J Histochem Cytochem 25: 135-147, 1977.

830. Russo, J., Wells, P. and Russo, I.H.: Adenosine triphosphatases as histochemical markers for cell of origin in experimental mammary carcinoma. Cancer Res 37: 1088-1098, 1977.

831. Saito, S., Terasaki, P.I. and Park, M.S.: Two loci determining B-lymphocyte specificities. Am J Clin Pathol (In Press).

832. Salzberg, S., Robin, M.S. and Green, M.: A possible requirement for protein synthesis early in the infectious cycle of the murine sarcoma-leukemia virus. Virology 76: 341-351, 1977.

833. Sarkar, N.H., Taraschi, N.E., Pomenti, A.A. and Dion, A.S.: Polypeptides of the mouse mammary tumor virus: II. Identification of two major glycoproteins with the viral structure. Virology 69: 677-690, 1976.

834. Sarma, P.S. and Log, T.: Defectiveness of a feline sarcoma virus demonstrable by the acquisition of a new envelope antigenic type by phenotypic mixing. Virology (In Press).

835. Sartorius, O.W. and Smith, H.S.: Contrast ductography for the recognition and localization of benign and malignant breast lesions: An improved technique. In Logar, W. (Ed.): Breast Carcinoma. New York, J. Wiley and Son (In Press).

836. Sartorius, O.W., Smith, H.S., Morris, P., Benedict, I. and Friesen, L.: Cytological evaluation of breast fluid in the detection of breast disease. J Natl Cancer Inst (In Press).

837. Sass, B., Peters, R.L. and Kelloff, G.J.: Differences in tumor incidence in two substrains of Claude BALB/c (BALB/cfCd) mice, emphasizing renal, mammary, pancreatic and synovial tumors. Lab Anim Sci 26: 736-741, 1976.

838. Schäfer, W. and Bolognesi, D.P.: Mammalian C-type oncornaviruses. Relationships between viral structural and cell surface antigens and their possible significance in immunological defense mechanisms. In Rapp, F. and Hanna, M.G., Jr. (Ed.): Contemporary Topics in Immunobiology. New York, Plenum Press, 1977 (In Press).

839. Schäfer, W., Claviez, M., Frank, H., Hunsman, G., Mcennig, V., Schwarz, H., Thiel, H.J., Bolognesi, D.P., Green, F.W., Langlois, A.J., Fischinger, P.J. and de Noronha, F.: Mammalian C-type oncorna viruses. Relationships between structural virus and cell surface and their possible significance in immunological defense mechanisms. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 88-96.

840. Schäfer, W., Fischinger, P.J., Collins, J.J. and Bolognesi, D.P.: Role of carbohydrate in biological functions of Friend murine leukemia virus gp71. J Virol 21: 35-40, 1977.

841. Schäfer, W., Schwarz, H., Thiel, H.J., Wecker, E. and Bolognesi, D.P.: Properties of mouse leukemia viruses. XIII. Serum-therapy of virus-induced murine leukemias. Virology 75: 401-418, 1976.

842. Schaffer, P.A., Bone, D.R. and Courtney, R.J.: DNA-negative temperature-sensitive mutants of herpes simplex virus type 1: Patterns of viral DNA synthesis following temperature shift-up. J Virol (In Press).

843. Schaffer, P.A. and Courtney, R. J.: Genetics of herpesvirus. In Animal Virology. New York, Academic Press, Inc., 1976 p 595-617.

844. Schaffhausen, B.S. and Benjamin, T.: Deficiency in histone acetylation in non-transforming host range mutants of polyoma virus. Proc Natl Acad Sci USA (In Press).

845. Schaller, J.P., Hoover, E.A. and Olsen, R.G.: Active and passive immunization of cats with inactivated feline oncornavirus. J Natl Cancer Inst (In Press).

846. Schaller, J.P., Hoover, E.A. and Olsen, R.G.: Differential immune response of newborn and adult cats to inactivated infectious feline oncornavirus. J Natl Cancer Inst (In Press).

847. Schaller, J.P., Milo, G.E., Blakeslee, J.R., Elliot, A., Olsen, R.G. and Yohn, D.S.: Influence of glucocorticoid, estrogen and androgen hormones on transformation of human cell in vitro by feline sarcoma virus. Cancer Res 36: 1980-1987, 1976.

848. Schechter, I., Burstein, Y. and Spiegelman, S.: Structure and function of immunoglobulin genes and immunoglobulin precursors. Annales D'Immunologie 127: 421-435, 1976.

849. Schidlovsky, G.: Structure of RNA tumor viruses. In Gallo, R.C. (Ed.): Recent Advances in Cancer Research. Cleveland, Ohio, CRC Press, Inc., 1976 (In Press).

850. Schlesinger, M. and Bekesi, J.G.: Natural autoantibodies cytotoxic for thymocytes in the sera of normal and leukemic AKR mice. J Natl Cancer Inst 1977 (In Press).

851. Schlom, J., Colcher, D., Drohan, W. and Kettmann, R.: The use of molecular hybridization to track the mode of transmission and distribution of murine mammary tumor viruses: A model for etiologic studies in human breast cancer. In Ito, Y. (Ed.): Progress in Experimental Tumor Research, Volume on "Viruses and Human Cancers". Basel, S. Karger AG (In Press).

852. Schlom, J., Colcher, D., Drohan, W., Kettmann, R., Michalides, R., Vlahakis, G. and Young, J.: Differences in mouse mammary tumor viruses: Relationship to early and late occurring mammary tumors. Cancer (In Press).

853. Schlom, J., Colcher, D., Drohan, W., Kimball, P., Michalides, R. and Schochetman, G.: Biochemical characterization of the mouse mammary tumor viruses and related isolates: Mason-Pfizer virus and the BudR-induced guinea pig virus. European Organization for Research on Treatment of Cancer: First Breast Cancer Working Conference. New York, Raven Press, 1976 (In Press).

854. Schlom, J., Colcher, D., Drohan, W., Kimball, P., Michalides, R., Schochetman, G. and Vlahakis, G.: Propagation and analysis of mouse mammary tumor viruses from different strains. In Schlessinger, D. (Ed.): Microbiology. Washington, D.C., American Society for Microbiology (In Press).

855. Schlom, J., Colcher, D., Drohan, W. and Schochetman, G.: Nucleic acid and protein studies of Mason-Pfizer virus. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 134-140.

856. Schlom, J., Michalides, R., Perk, K., Pearson, J.W. and Dahlberg, J.E.: Biochemical properties of the B-type retravirus of guinea pig and an agent in the plasma of guinea pigs with L2C leukemia. Fed Proc (In Press).

857. Schlom, J., Schochetman, G., Michalides, R., Kimball, P., Drohan, W. and Colcher, D.: Characterization of the RNA genomes and proteins of the mouse mammary tumor viruses, the Mason-Pfizer virus, and the BudR-induced guinea pig virus. In Gallo, R.C. (Ed.): Molecular Biology of Neoplasia: Current Focus of Cancer Research. (In Press).

858. Schneider, R.: Comparative epidemiological aspects of naturally occurring malignant lymphoma in domestic cats and rhesus monkeys. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

859. Schochetman, G., Boehm-Truitt, M. and Schlom, J.: Antigenic analysis of the major structural protein of the Mason-Pfizer monkey virus. J Immunol 117: 168-173, 1976.

860. Schochetman, G. and Schlom, J.: Independent polypeptide chain initiation sites for the synthesis of different classes of proteins for an RNA tumor virus: Mouse mammary tumor virus. Virology 73: 431-441, 1976.

861. Schochetman, G. and Schlom, J.: RNA tumor viruses and breast cancer. In St-Arneault, G., Band, P. and Israel (Ed.): Recent Results in Cancer Research. Breast Cancer: A Multidisciplinary Approach. New York, Springer-Verlag, Vol 57, p 21-25.

862. Schultz, R.M., Chirigos, M.A., Mohr, S.J. and Woods, W.A.: Tumoricidal effect in vitro of peritoneal macrophages from mice treated with pyran copolymer. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 437-448.

863. Schultz, R.M., Papamatheakis, J.D. and Chirigos, M.A.: Correlation between anti-tumor activity and macrophage activation by polyanions. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

864. Schultz, R.M., Papamatheakis, J.D. and Chirigos, M.A.: Direct activation in vitro of mouse peritoneal macrophages by pyran copolymer (NSC 46015). Cell Immunol 29: 55-61, 1977.

865. Schultz, R.M., Papamatheakis, J.D. and Chirigos, M.A.: Tumoricidal effect in vitro of peritoneal macrophages from mice treated with glucan. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977 (In Press).

866. Schultz, R.M., Papamatheakis, J.D. and Chirigos, M.A.: Interferon, the ultimate inducer of macrophage activation by polyanions. Science (In Press).

867. Schultz, R.M., Papamatheakis, J.D., Luetzeler, J., Ruiz, P. and Chirigos, M.A.: Macrophage involvement in the protective effect of pyran copolymer against the Madison lung carcinoma (109). Cancer Res 37: 358-364, 1977.

868. Schultz, R.M., Papamatheakis, J.D., Stylos, W.A. and Chirigos, M.A.: Augmentation of specific macrophage-mediated cytotoxicity: Correlation with agents which enhance antitumor resistance. Cell Immunol 25: 309-316, 1976.

869. Schultz, R.M., Ruiz, P., Chirigos, M.A. and Heine, U.: Establishment and characterization of a cell line derived from a spontaneous murine lung carcinoma (M109). In Vitro (In Press).

870. Schultz, R.M., Ruiz, P., Chirigos, M.A., Heine, U. and Nelson-Rees, W.A.: Establishment and characterization of a cell line derived from spontaneous murine lung carcinoma (M109). In Vitro (In Press).

871. Schultz, R.M., Woods, W.A., Mohr, S.J. and Chirigos, M.A.: Immune response of CD2F1 mice to a tumor allograft during pyran copolymer-induced tumor enhancement. Cancer Res (In Press).

872. Schwartz, D.E., Zamecnik, P.C. and Weith, H.L.: The Rous sarcoma virus genome is terminally redundant: The 3' sequence. Proc Natl Acad Sci USA 74: 994-998, 1977.

873. Scolnick, E.M., Goldberg, R.J., Williams, D. and Parks, W.P.: Characterization of rat genetic sequences of Kirsten sarcoma virus: A distinct class of endogenous rat type C viral sequences. J Virol (In Press).

874. Scolnick, E.M., Williams, D., Maryak, J., Vass, W.C., Goldberg, R.J. and Parks, W.P.: Type C particle-positive and type C particle-negative rat cell lines: Characterization of the coding capacity of endogenous sarcoma virus-specific RNA. J Virol 20: 570-582, 1976.

875. Scolnick, E.M., Williams, D. and Parks, W.P.: Purification and characterization of viral RNA of a sarcoma virus isolated from a Woolly monkey. Nature 264: 23-30, 1976.

876. Seebeck, T., Shaw, J.E. and Pagano, J.S.: Synthesis of Epstein-Barr virus DNA in vitro: Effects of phosphonoacetic acid, N-ethylmaleimide, and ATP. J Virol 21: 435-438, 1977.

877. Seigneurin, J.M., Desgranges, C., Lavoué, M.F. and de Thé, G.: Herpes simplex Raji (A44), a new cell line for serologic testing by immunofluorescence. J Immunol 117: 950-952, 1976.

878. Seman, G. and Dmochowski, L.: Methods for electron microscopy of viruses. In Busch, H. (Ed.): Methods in Cancer Research. New York, Academic Press, Inc., Vol XII, 1975 (In Press).

879. Sen, A., Sherr, C.J. and Todaro, G.J.: Specificity of in vitro binding of primate type C viral RNA and the homologous viral p12 core protein. Science 193: 326-328, 1976.

880. Sen, A., Sherr, C.J. and Todaro, G.J.: Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. Cell 10: 489-496, 1977.

881. Sen, A. and Todaro, G.J.: The genome-associated specific RNA binding proteins of avian and mammalian type C viruses. Cell 10: 91-99, 1977.

882. Sesterhenn, K., Krueger, G.R., Uhlmann, C.H., Ablashi, D.V., Samii, H., Wustrow, F. and Fischer, R.: Klassifikation maligner lymphome des Halsbereiches: Kombinierte morphologische, immunzytologische, sero-logische und zelkluruntersuchingen laryng. Rhinol Otologie 55: 823-832, 1976.

883. Shapiro, S.Z. and August, J.T.: The use of immunoprecipitation to study the synthesis and cleavage processing of viral proteins. J Immunol Method 13: 153-159, 1976.

884. Shapiro, S.Z. and August, J.T.: Proteolytic cleavage events in oncornavirus protein synthesis. Biochim Biophys Acta 458: 375-396, 1976.

885. Shapiro, S.Z., Strand, M. and August, J.T.: High molecular weight precursor polypeptides to structural proteins of Rauscher murine leukemia virus. J Mol Biol 107: 459-477, 1976.

886. Shapiro, S.Z., Strand, M. and Billiau, A.: Synthesis and cleavage processing of oncornavirus proteins during interferon inhibition of virus particle release. Infection & Immunity (In Press).

887. Shaw, J.E., Seebeck, T., Li, J.-L.H. and Pagano, J.S.: The Epstein-Barr virus DNA synthesized in superinfected Raji cells. Virology (In Press).

888. Sheffield, J.B. and Daly, T.M.: Extrinsic labeling of MuMTV with a galactose oxidase-tritiated borahydide method. Virology (In Press).

889. Sheffield, J.B., Daly, T.M., Dion, A.S. and Taraschi, N.E.: Procedures for radioimmunoassay of the mouse mammary tumor virus. Cancer Res (In Press).

890. Sheffield, J.B., Zacharchuk, C.M., Taraschi, N.E. and Daly, T.M.: Effect of trypsin on mouse mammary tumor virus. J Virol 19: 255-266, 1976.

891. Shen, F.W.: Preparation of Lyt antisera. Immunogenetics (In Press).

892. Sherr, C.J. and Benveniste, R.E.: Evolution and interspecies transfer of endogenous type C viral genes. 5th Reunion du Groups D'Etudes des Oncornaviruses. Centre "les Fontaines", Chantilly, France, 1976.

893. Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Todaro, G.J.: Type C viruses from Kirsten sarcoma-transformed mink cells cocultivated with primate cells and expressing p30 antigens related to feline leukemia virus. J Virol 19: 346-358, 1976.

894. Shih, T.Y. and Khoury, G.: Isolation of viral specific RNA from SV40-infected cells by viral DNA chemically linked to a cellulose matrix. Biochemistry 15: 487-492, 1976.

895. Shih, T.Y., Young, H.A., Parks, W.P. and Scolnick, E.M.: In vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin: Elongation of chromatin associated RNA by E. coli RNA polymerase. Biochemistry (In Press).

896. Shlomai, J., Asher, Y. and Becker, Y.: In vitro synthesis of herpes simplex virus DNA in nuclei isolated from infected BSC-1 cells. J Gen Virol 34: 223-324, 1977.

897. Shlomai, J., Friedmann, A. and Becker, Y.: Replicative intermediates of herpes simplex virus DNA. Virology (In Press).

898. Shlomai, J., Strauss, B., Asher, Y., Friedmann, A. and Becker, Y.: Analysis of herpes simplex virus DNA synthesized in infected nuclei by chromatography on benzoylated naphthoylated DEAE cellulose columns. J Gen Virol 32: 189-204, 1976.

899. Shore, S., Melewicz, F., Milgrom, H. and Nahmias, A.: Antibody dependent cell-mediated cytotoxicity to target cells infected with herpes simplex viruses. In Friedman, H., Escobar, M. and Reichard, M. (Ed.): The Reticular and Endothelial Systems in Health and Diseases: Immunological and Pathological Aspects. Adv Exp Med Biol 73B, 1976.

900. Shore, S., Milgrom, H., Wood, P.A. and Nahmias, A.: Efficacy of cord blood mononuclear cells in antibody-dependent cell-mediated cytotoxicity to target cells infected with herpes simplex virus. Pediatrics 59: 22-28, 1977.

901. Shustik, C., Cohen, I.R., Schwartz, R.S. and Latham-Griffin, E.: T lymphocytes with promiscuous cytotoxicity. Nature 263(5579): 699-701, 1976.

902. Sigel, M.M., Lopez, D.M. and Ortiz-Muniz, G.: In vitro immune responses to viral and tumor antigens in murine breast cancer. Cancer Res 36: 748-752, 1976.

903. Sigel, M.M., Lopez, D.M. and Ortiz-Muniz, G.: Migration inhibition and blastogenic reactions with viral and tumor antigens in transplantable mammary tumor systems. Ann NY Acad Sci 276: 358-368, 1976.

904. Silberstein, H. and August, J.T.: Characterization of a virion protein kinase as a virus-specified enzyme. J Biol Chem (In Press).

905. Silberstein, H. and August, J.T.: Purification and properties of a virion protein kinase. J Biol Chem (In Press).

906. Silverstone, A.E., Cantor, H., Goldstein, G. and Baltimore, D.: Terminal deoxynucleotidyl transferase is found in prothymocytes. J Exp Med 144: 543-548, 1976.

907. Simonds, J.A., Ebert, P.S., Graham, B.J. and Vande Woude, G.F.: The effect of multiplicity of infection on the DNA species synthesized in Herpesvirus saimiri. Bact Proc (In Press).

908. Simons, M.J., Chan, S.H., Ho, J.H.C., Chau, J.C.W., Day, N.E. and De The', G.: A Singapore-2 associated LD antigen in Chinese patients with nasopharyngeal carcinoma. In Histocompatibility Testing. Copenhagen, Munksgaard, 1975 (In Press).

909. Simons, M.J. and Day, N.E.: HLA patterns and NPC. In Henderson, B.E. (Ed.): Proc Symp on Epidemiology and Cancer Registries in the Pacific Basin, Maui, Hawaii, Nov 1975. Los Angeles, Ca., USC Press (In Press).

910. Simons, M.J., Wee, G.B., Goh, E.H., Chan, S.H., Sharmugaratnam, K., Day, N.E. and De The', G.: Immunogenetic aspects of nasopharyngeal carcinoma (NPC): IV. Increased risk in Chinese for NPC associated with a Chinese-related HLA profile (A2, Sin2). J Natl Cancer Inst (In Press).

911. Simons, M.J., Wee, G.B., Singh, D., Dharmalingam, S., Yong, N.K., Chau, J.C.W., Ho, J.H.C., Day, N.E. and De The, G.: Immunogenetic aspects of nasopharyngeal carcinoma (NPC): V. Confirmation of a Chinese-related HLA profile (A2, Singapore 2) associated with an increased risk in Chinese for NPC. In Henderson, B.E. (Ed.): Proc Symp on Epidemiology and Cancer Registries in the Pacific Basin, Maui, Hawaii, Nov 1975. Los Angeles, Ca., USC Press (In Press).

912. Singh, I., Hatheway, J.M., Tsang, K.Y., Blakemore, W.S. and McAllister, R.M.: An animal model for human osteosarcoma. Surgery 81: 168-175, 1977.

913. Singh, M., Singh, V.N., August, J.T. and Horecker, B.L.: Changes in activities of key enzymes of glucose metabolism in chick-embryo fibroblasts during growth. Journal de Microscopie et de Biologie Cellulaire (In Press).

914. Sinkovics, J.G., Kay, H.D. and Thota, H.: The evaluation of chemoimmunotherapy regimens by in vitro lymphocyte cytotoxicity directed to cultured human tumor cells. Bibliotheca Haematologica. New York, S. Karger (In Press).

915. Siu, C.-H., Lerner, R.A., Firtel, R.A. and Loomis, W.F.: Changes in plasma membrane proteins during development of Dictyostelium discoideum. In McMahon, D. and Fox, F. (Ed.): Developmental Biology, Vol 2. Menlo Park, California, W.A. Benjamin Co., Inc., 1976, p 129-134.

916. Siu, C.-H., Lerner, R.A. and Loomis, W.F.: Rapid accumulation and disappearance of plasma membrane proteins during development of wild-type and mutant strains of Dictyostelium discoideum. J Mol Biol (In Press).

917. Skelly, J., Gacto, M., Steiner, M.R. and Steiner, S.: Preliminary evidence for novel fucose-containing lipids in normal and murine sarcoma virus-transformed rat cells. Biochem Biophys Res Commun (In Press).

918. Sliski, A.H., Essex, M., Meyer, C. and Todaro, G.J.: Feline oncornavirus associated cell membrane antigen (FOCMA): Expression in feline sarcoma virus transformed non-producer mink cells. Science 1977 (In Press).

919. Smith, E.J., Stephenson, J.R., Crittenden, L.B. and Aaronson, S.A.: Avian leukosis-sarcoma virus gene expression: Noncoordinate control of group specific antigens in virus negative avian cells. Virology (In Press).

920. Smith, G.C., Helmke, R.J., Heberling, R.L., Barker, S.T. and Kalter, S.S.: Oncornavirus-like particles in baboon type C virus infected chimpanzee lung cells (SFRE:CL-1). J Natl Cancer Inst (In Press).

921. Smith, G.C., Kalter, S.S., Heberling, R.L. and Helmke, R.J.: Particles resembling mouse hepatitis virus in nude mouse uterus. J Virol (In Press).

922. Smith, H.S., Owens, R.B., Hiller, A.J., Nelson-Rees, W.A. and Johnston, J.C.: The biology of human cells in tissue culture I: Characterization of cells derived from osteogenic sarcomas. Int J Cancer 17: 219-234, 1976.

923. Smith, H.S., Owens, R.B., Nelson-Rees, W.A., Springer, E.L., Dollbaum, E.M., Hackett, A.J. and Williams, R.: Epithelial cell from human carcinomas. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

924. Smith, H.S., Riggs, J.L. and Springer, E.L.: Expression of antigenic crossreactivity to RD114 p30 protein in a human fibrosarcoma cell line. Proc Natl Acad Sci USA 74: 744-748, 1977.

925. Smith, H.S., Turner, S., Leong, J.C. and Rigby, P.W.J.C.: Effect of passage in culture on a clone of BALB/c3T3 cells transformed by simian virus 40. J Virol 19: 146-153, 1976.

926. Smith, P.G. and Pike, M.C.: Current epidemiological evidence for transmission of Hodgkin's disease. Cancer Res 36: 660-662, 1976.

927. Smith, P.G. and Pike, M.C.: Generalization of two tests for the detection of household aggregation of diseases. Biometrics 32: 817-828, 1976.

928. Smith, P.G., Pike, M.C., Till, M.M. and Hardistry, P.M.: Epidemiology of childhood leukemia in greater London: A search for evidence of transmission assuming a possibly long latent period. Br J Cancer (In Press).

929. Soeiro, R., Sveda, M.M., Krontiris, T.G. and Ray, U.: Host restriction of Friend virus: Proteins and messenger RNA. In Baltimore, D., Huang, A.S. and Fox, C.F. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976, p 257-268.

930. Sonis, S.T., Stelos, P., Stylos, W.A. and Wilson, R.E.: Inhibition of lymphoma cell proliferation by supernatant from fibro-sarcoma cultures: Preliminary evidence that the inhibitory material is Prostaglandin E. Prostaglandins 13: 87-96, 1977.

931. Sonstegard, R.A. and Papas, T.S.: Descriptive and comparative studies of type C virus DNA polymerase of fish. Am Assoc Cancer Res (In Press).

932. Sottong, P., Harris, R., Graham, B., Rupp, R., Bell, C., Conlon, T. and Klecker, J.: Purification of herpesvirus nucleocapsids by fluorocarbon extraction. J Virol (In Press).

933. Sottong, P., Harris, R., Graham, B., Rupp, R., Bell, C., Conlon, T. and Klecker, J.: Purification of herpesvirus nucleocapsids by fluorocarbon extraction. Microbios (In Press).

934. Sottong, P., Rankin, C.T., Kramarsky, B., Harris, R., Bell, C., Klecker, J. and Vande Woude, G.F.: Purification of herpes viruses on renografin gradients. J Virol (In Press).

935. Spiegelman, S.: Evidence for viruses in human neoplasias. Hematology (In Press).

936. Spiegelman, S.: The search for viruses in human cancer. In Richards, M.A. (Ed.): Proc Am Philosophical Soc Symp, Philadelphia, Pa. 120: 69-86, 1976.

937. Spiegelman, S.: Molecular virology of cancer. Proc Int Assoc for the Promotion of Clinical and Experimental Research in Medicine, Vienna, Oct 1974 (In Press).

938. Spiegelman, S.: Molecular virology of cancer. In Deutsch, E., Moser, K., Rainer, H. and Stacher, A. (Ed.): Molecular Base of Malignancy. Stuttgardt, Germany, Georg Thieme Publishers, 1976, p 178-197.

939. Spiegelman, S.: Molecular evidence for the association of RNA tumor viruses with human mesenchymal malignancies. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977, p 391-429.

940. Spiegelman, S.: Molecular approach to the etiology of human cancer. Pure Appl Chem 47: 11-23, 1976.

941. Spiegelman, S.: The etiology of human cancer viewed as a problem in molecular genetics. In: Biological Signals. Sweden, Royal Physiographical Society of Lund, Sweden. (In Press).

942. Springer, E.L., Hackett, A.J. and Nelson-Rees, W.A.: Alteration of the cell membrane architecture during suspension and monolayer culturing. Int J Cancer (In Press).

943. Springer, E.L., Hackett, A.J. and Nelson-Rees, W.A.: Dynamics of lamelloplasm formation during cell attachment to the growth surface. Int J Cancer (In Press).

944. Springer, E.L., Riggs, J.L. and Hackett, A.J.: Scanning electron micrographs. In Wistreich, G.A. (Ed.): Concepts in Biology. New York, Glencoe Press (In Press).

945. Springer, E.L. and Roth, I.L.: Freeze Etch Micrographs. In Sutherland, I.W. (Ed.): Bacterial Exopolysaccharides. New York, Academic Press, Inc. (In Press).

946. St. Jeor, S.C. and Hutt, R.: Cell DNA replication as a function in the synthesis of human cytomegalovirus. J Gen Virol 1977 (In Press).

947. St. Jeor, S.C. and Weisser, A.: Persistence of cytomegalovirus in human lymphoblasts and peripheral leukocyte cultures. Infection & Immunity 15: 402-409, 1977.

948. Staneloni, R.J., Fluck, M.M. and Benjamin, T.L.: Host range selection of transformation defective "Hr-t" mutants of polyoma virus. Virology 1977 (In Press).

949. Stehelin, D., Varmus, H.E., Bishop, J.M. and Vogt, P.K.: DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian cells. Nature (In Press).

950. Steiner, S. and Steiner, M.R.: Glycolipids in virus-transformed cells. In Nicolau, C. (Ed.): Virus-Transformed Cell Membranes. New York, Academic Press, Inc. (In Press).

951. Steiner, S. and Steiner, M.R.: Fucolipid patterns of highly and weakly tumorigenic simian virus 40 and herpes simplex virustransformed cell lines. Intervirology 6: 32-41, 1976.

952. Stephens, R., Traul, K., Gaudrau, P., Yeh, J., Fisher, L. and Mayyasi, S.A.: Comparative studies on EBV antigens by immuno-fluoresence and immunoperoxidase techniques. Int J Cancer 19: 305-316, 1977.

953. Stephenson, J.R. and Aaronson, S.A.: Differential regulation of endogenous type C viruses of mouse cells. Microbiology (In Press).

954. Stephenson, J.R. and Aaronson, S.A.: Endogenous type C viral expression in primates. Nature (In Press).

955. Stephenson, J.R. and Aaronson, S.A.: Induction of an endogenous B-tropic type C RNA virus from SWR/J mouse embryo cells in tissue culture. Virology 70: 352-359, 1976.

956. Stephenson, J.R. and Aaronson, S.A.: Search for antigens and antibodies cross-reactive with type C viruses of the woolly monkey and gibbon ape in animal models and in humans. Proc Natl Acad Sci USA (In Press).

957. Stephenson, J.R., Barbacid, M., Tronick, S.R., Hino, S. and Aaronson, S.A.: Proteins of type C RNA tumor viruses. In Gallo, R.C. (Ed.): Recent Advances in Cancer Research. Cleveland, Ohio, CRC Press, Inc., 1976 (In Press).

958. Stephenson, J.R., Cabradilla, C. and Aaronson, S.A.: Genetic factors influencing endogenous type C RNA viruses of mouse cells: Control of viral polypeptide expression in the C57BL/10 strain. Intervirology 6: 258-269, 1976.

959. Stephenson, J.R., Essex, M., Hino, S., Hardy, W.D., Jr. and Aaronson, S.A.: Feline oncornavirus-associated cell membrane antigen (FOCMA). VII. Distinction between FOCMA and the major virion glycoprotein. Proc Natl Acad Sci USA (In Press).

960. Stephenson, J.R., Hino, S., Garrett, E.W. and Aaronson, S.A.: Immunologic cross-reactivity of Mason-Pfizer monkey virus with type C RNA viruses endogenous to primates. Nature 261: 609-611, 1976.

961. Stephenson, J.R., Peters, R.L., Hino, S., Donahoe, P.M., Long, L.K., Aaronson, S.A. and Kelloff, G.J.: Natural immunity in mice to structural polypeptides of endogenous type C RNA viruses. J Virol 19(2): 890-898, 1976.

962. Steuer, A.F., Rhim, J.S. and Ting, R.C.: Survival of human cells in the aggregate form: Potential index of in vitro cell transformation. J Natl Cancer Inst 1977 (In Press).

963. Strand, M. and August, J.T.: Purification and analysis of a Gross murine oncornavirus protein of about 12,000 daltons specific for the Gross virus subgroup. Virology (In Press).

964. Strand, M. and August, J.T.: Oncornavirus envelope glycoprotein in serum of mice. Virology 75: 130-144, 1976.

965. Strand, M. and August, J.T.: Protein homology of mammalian RNA tumor viruses. In Zhdanov, V.M. (Ed.): Cancer and Leukemia Viruses. Proc of Biology of Oncogenic Viruses of Animals and Man, 1975 (In Press).

966. Strand, M., August, J.T. and Croce, C.: Selective induction of murine oncornavirus gene expression in somatic cell hybrids between mouse peritoneal macrophages and SV-40-transformed human cells. Virology (In Press).

967. Strand, M., August, J.T. and Croce, C.: Oncornavirus genetic expression: Murine virus gene induction by human cell chromosomes. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

968. Strand, M., August, J.T. and Jaenisch, R.: Oncornavirus gene expression during embryonal development of the mouse. Virology (In Press).

969. Strickland, J.E., Saviolakis, G.A., Fowler, A.K., Kouttab, N.M. and Hellman, A.: Impaired estrogen-mediated production of type C viral DNA polymerase in aged NIH Swiss mouse uteri. Proc Soc Exp Biol Med 153: 63-69, 1976.

970. Strnad, B.C. and Aurelian, L.: Proteins of herpesvirus type 2: I. Virion, nonvirion and antigenic polypeptides in infected cells. Virology 73: 244-258, 1976.

971. Stulberg, C.S., Peterson, W.D., Jr. and Simpson, W.F.: Identification of cells in culture. Am J Hematol 1: 237-242, 1976.

972. Stulberg, C.S. and Simpson, W.F.: A reliable and rapid procedure for development of human fibroblastic cell strains. Tissue Culture Assoc Manual I(4): 1976.

973. Sugawara, K., Gilead, Z. and Green, M.: Purification and molecular characterization of adenovirus type 2 DNA binding protein. J Virol 21(1): 338-346, 1977.

974. Sugawara, K., Gilead, Z., Wold, W.S.M. and Green, M.: Immuno-fluorescence study of the adenovirus type 2 single stranded DNA binding protein in infected and transformed cells. J Virol (In Press).

975. Summers, W.C. and Klein, G.: Inhibition of Epstein-Barr virus DNA synthesis and late gene expression by phosphonoacetic acid. J Virol 18(1): 151-155, 1976.

976. Sveda, M.M., Fields, B.N. and Soeiro, R.: Fate of input virion RNA - biological studies. J Virol (In Press).

977. Takasugi, M.: Specificity and effector-target interaction. Natl Cancer Inst Monogr (In Press).

978. Takasugi, M., Akira, D., Mullen, Y. and Takasugi, J.: Natural cell-mediated cytotoxicity in Man. In Proc 3rd Int Symp on Detection and Prevention of Cancer, Apr 1976, New York (In Press).

979. Takasugi, M., Akira, D., Takasugi, J. and Mickey, M.R.: Specificities in human natural cell-mediated cytotoxicity. J Natl Cancer Inst (In Press).

980. Takasugi, M., Koide, Y., Akira, D. and Ramseyer, A.: Specificities in natural cell mediated cytotoxicity by the cross competitition assay. Int J Cancer (In Press).

981. Takasugi, M., Kuichi, M. and Opelz, M.: Lymphocyte interactions in autologous and allogeneic reaction. Transplant Proc 9: 789-792, 1977.

982. Takasugi, M. and Mickey, M.R.: Interaction analysis of selective and nonselective cell-mediated cytotoxicity. J Natl Cancer Inst 57: 255-261, 1976.

983. Takasugi, M., Ramseyer, A. and Takasugi, J.: Decline in natural cell-mediated cytotoxicity in patients with tumor progression. Cancer Res 37: 413-418, 1977.

984. Takeichi, N. and Boone, C.W.: Spontaneous rosette formation of rat thymus cells with guinea pig erythrocytes. Cell Immunol 27: 52-59, 1976.

985. Takeichi, N., Boone, C.W., Halden, H.T. and Herberman, R.B.: Immunological study of two stocks of Moloney sarcoma virus producing regressive and progressive tumors in C57BL/6 mice. J Immunol (In Press).

986. Takeichi, N., Boone, C.W. and Klein, E.: Local adoptive transfer to mice of human delayed hypersensitivity reactions by the radioisotopic footpad assay. Clin Exp Immunol 26: 310-314, 1976.

987. Takeichi, N., Economou, C.K. and Boone, C.W.: Accelerated regeneration of trypsin-treated surface antigens of SV40 transformed BALB/3T3 cells induced by X-irradiation. Cancer Res 36: 1258-1262, 1976.

988. Takeichi, N., Economou, C.K. and Boone, C.W.: Local adoptive transfer of anti-tumor cellular immunity to xenogeneic animals studied with a radioisotopic footpad assay. J Natl Cancer Inst 56: 131-134, 1976.

989. Takeichi, N., Kodama, T., Kobayashi, H. and Boone, C.W.: Adoptive immunotherapy of Gross virus producing lymphomas and methylcholanthrene-induced fibrosarcomas in tolerant rats. Cancer Res 36: 4039-4043, 1976.

990. Takeichi, N., Oikawa, T. and Boone, C.W.: Delayed type hyper-sensitivity reactions to tumor-associated antigens in syngeneic and allogeneic mice. Cancer Res (In Press).

991. Tamerius, J., Nepom, J.T., Hellström, I. and Hellström, K.E.: Tumor associated blocking factors: Isolation from sera of tumor-bearing mice. J Immunol (In Press).

992. Tanaka, A.: Suitability of Epstein-Barr virus DNA obtained from superinfected Raji cells for complementary RNA hybridization studies. J Gen Virol 34(2): 375-379, 1977.

993. Tanaka, A., Miyagi, M., Yajima, Y. and Nonoyama, M.: Improved production of Epstein-Barr virus DNA for nucleic acid hybrid-ization studies. Virology 74: 81-85, 1976.

994. Tanaka, A., Nonoyama, M. and Hampar, B.: Partial elimination of latent Epstein-Barr virus genomes from virus producing cells by cycloheximide. Virology (In Press).

995. Tavitian, A., Larsen, C.J. and Boiron, M.: Sequences detected in the RNA of human leukemic cells by the c-DNA probes. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Leukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977 (In Press).

996. Taylor, B.A., Bedigian, H.G. and Meier, H.: Genetic studies of the Fv-1 locus of the mouse: Linkage with Gpd-1 in recombinant inbred lines. J Virol (In Press).

997. Taylor, B.A. and Meier, H.: Mapping the adrenal lipid depletion gene of the AKR/J mouse strain. Genet Res (In Press)

998. Tennant, R.W.: Inhibition of murine RNA tumor virus expression and tumorigenesis by polyadenylic acids. Natl Cancer Inst Morogr (In Press)

999. Tennant, R.W.: Inhibition of murine RNA tumor virus expression and tumorigenesis by polyadenylic acids. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 157-162.

1000. Tennant, R.W., Rascati, R.J. and Lavelle, G.C.: Mechanisms in endogenous leukemia virus induction by radiation and chemicals. In Duplan, J.F. (Ed.): Radiation-Induced Leukemogenesis and Related Viruses. Amsterdam, The Netherlands, Elsevier Scientific Publishing, 1977 (In Press).

1001. Tennant, R.W., Schulter, B., Myer, F.E., Otten, J.A., Yang, W.K. and Brown, A.: Genetic evidence for a product of the Fv-1 locus that transfers resistance to mouse leukemia viruses. J Virol 20: 589-596, 1976.

1002. Terada, M., Fried, J., Nudel, U., Rifkind, R.A. and Marks, P.A.: Transient inhibition of initiation of S-phase associated with induction of murine erythroleukemia cells to erythroid differ-entiation by dimethylsulfoxide. Proc Natl Acad Sci USA 74(1): 248-252, 1977.

1003. Teramoto, Y.A., Cardiff, R.D. and Lund, J.K.: The structure of the mouse mammary tumor virus: Isolation and characterization of the core. Virology (In Press).

1004. Tevethia, S.S. and Rapp, F.: Comparative immunology of carcinogenesis by DNA viruses. In Hanna, M.G., Jr. and Rapp, F. (Ed.): Immunobiology of Oncogenic Viruses. In Contemporary Topics in Immunobiology. New York, Plenum Press, 1977, Vol 6, p 1-69.

1005. Timbury, M.C., Hendricks, M.I. and Schaffer, P.A.: Collaborative study of temperature-sensitive mutants of herpes simplex virus type 2. J Virol 18: 1139-1142, 1976.

1006. Todaro, G.J.: RNA tumor virus genes (virogenes) and the transforming genes (oncogenes): Genetic transmission, infectious spread, and modes of transmision. J Natl Cancer Inst 1977 (In Press).

1007. Todaro, G.J.: RNA tumor virus genes (virogenes) and the trans-forming genes (oncogenes): Genetic transmission, infectious spread and modes of transmission. In Hiatt, H.H., Watson, J.D. and Winston, J.A. (Ed.): Origins of Human Cancer. Symp on Quant Biol, Cold Spring Harbor Laboratory, New York, May 1976, Vol 40 (In Press).

1008. Todaro, G.J.: Evidence for the interspecies transmission of tumor virus genes. In Gallo, R.C. (Ed.): Advances in Cancer Research: Cell Biology Molecular Biology and Tumor Virology. Cleveland, Ohio, CRC Press, Inc. (In Press).

1009. Todaro, G.J.: Type C virogeres: Modes of transmissicn and evoluationary aspects. In Neth, R., Gallo, R.C., Mannweiler, K. and Moloney, W.C. (Ed.): Modern Trends in Human Ieukemia II. Biochemical, Biological and Immunological Aspects of Cell Differentiation. Munchen, J.F. Lehmanns Verlag, 1977, p 357-374.

1010. Todaro, G.J., Benveniste, R.E. and Sherr, C.J.: Interspecies transfer of RNA tumor virus genes: Implications for the search of "human" type C viruses. In Baltimore, D., Huang, A.S. and Fox, C.E. (Ed.): Animal Virology. ICN-UCLA Symp on Molecular and Cellular Biology, Vol 4. New York, Adacemic Press, Inc., 1976, p 369-384.

1011. Todaro, G.J., DeLarco, J. and Cohen, S.: Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor (EGF) to cells. Nature 264: 26-31, 1976.

1012. Todaro, G.J., Sherr, C.J. and Benveniste, R.E.: Baboons and their close relatives are unusual among primates in their ability to release complete endogencus type C viruses. Virology 72: 278-282, 1976.

1013. Tronick, S.R., Golub, M.M., Stephenson, J.R. and Aaronson, S.A.: Distribution and expression in mammals of genes related tc an endogenous type C RNA virus of Cdoccileus hemionus. J Virol (In Press).

1014. Troxler, D.H., Boyars, J.K., Parks, W.P. and Scolnick, E.M.: Friend strain of spleen focus forming virus: A recombinant between mouse ecotropic viral sequences and sequences related tc xenotropic virus. J Virol 1977 (In Press).

1015. Troxler, D.H., Parks, W.P., Vass, W.C. and Scolnick, E.M.: Isolation of a fibroblast nonproducer cell line containing the Friend strain of spleen-focus forming virus. Virology 76: 602-615, 1977.

1016. Tucker, H.S., Weens, J., Tsiblis, P., Schwartz, R.S., Khiroya, R. and Donnelly, J.: Influence of H-2 complex on susceptibility tc infection by murine leukemia virus. J Immunol (In Press).

1017. Twardzik, D.R., Ablashi, D.V., Easton, J.M. and Armstrong, G.R.: Soluble complement-fixing antigens in Epstein-Barr virus cell systems. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 412-416.

1018. Vaidya, A.B., Lasfargues, E.Y., Heubel, G., Lasfargues, J.C. and Moore, D.H.: Murine mammary tumor virus: Characterization of infection of non-murine cells. J Virol 18: 911-917, 1976.

1019. Valsamis, M.P., Levine, P.H., Rapin, I., Santorineou, M. and Shulman, K.: Primary intracranial Burkitt's lymphoma in an infant. Cancer (In Press).

1020. Vande Woude, G.F., Robey, W.G., Oskarsson, M.K., Haapala, D.K., Fischinger, P.J., Naso, R.B. and Arlinghaus, R.B.: Properties of Moloney sarcoma virus-specific p60 and its detection in transformed cells. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 125-127.

1021. Vanky, F., Klein, E., Cornain, S., Bakacs, T., Stjernswärd, J. and Nilsonne, U.: Search for anti-tumor response in a bone tumor patient with a long clinical history. Int J Cancer 18: 282-292, 1976.

1022. Van Nie, R. and Hilgers, J.: Genetic analysis of mammary tumor induction and expression of mammary tumor virus artigen in hormone-treated ovariectomized GR mice. J Natl Cancer Inst 56(1): 2732, 1976.

1023. Van Pelt, F.G. et al: Lack of interference in antigen production in vitro between B-type and C-type oncornaviruses as determined by double strain immunofluorescence. Eur J Cancer 12: 321, 1976.

1024. Varmus, H.E., Heasley, S., Linn, J. and Wheeler, K.: Use of alkaline sucrose gradients in a zonal rotor to detect integrated and unintegrated avian sarcoma virus-specific DNA in cells. J Virol (In Press).

1025. Varmus, H.E. and Shank, P.: Unintegrated viral DNA is synthesized in the cytoplasm of avian sarcoma virus transformed duck cells by viral DNA polymerase. J Virol (In Press).

1026. Vasa-Thomas, K.A., Bellomy, E.B. and Coggin, J.H., Jr.: Characterization of immune responses to spontaneous hamster lymphomas. J Natl Cancer Inst 1977 (In Press).

1027. Verma, I.M. and Hunter, E.: Reverse transcriptase is coded by the viral RNA. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

1028. Verma, R.S. and Dosik, H.: Fluorescent microscopy of human chromosomes. 15th Somatic Cell Genetics Conf, Virginia, 1976 (In Press).

1029. Verma, R.S., Dosik, H. and Ehlin, M.: An improved method for photographing fluorescent human chromosomes. J Microsc 108(3): 339-341, 1977.

1030. Verma, R.S., Dosik, H. and Lubs, H.A.: Frequency of RFA color polymorphisms of human acrocentric chromosomes in Caucasians: Interrelationships with QFQ polymorphisms. Ann Hum Genet 1977 (In Press).

1031. Verma, R.S., Dosik, H. and Wexler, I.B.: Inherited pericentric inversion of chromosome No. 2 with Robertsonian translocation resulting in trisomy for chromosome 13q+. J Genet Hum 1977 (In Press).

1032. Verma, R.S., Rubenstein, C. and Dosik, H.: Effect of Co2 on short term human lymphocyte culture in vitro. In Vitro 1976 (In Press).

1033. Vernon, M.L., Knipscher, R.C., More, N.S., Ruch, D.G., Green, I. and Rhim, J.S.: Morphology of the guinea pig leukemia associated viruses. Fed Proc 1977 (In Press).

1034. Vernon, M.L. and Price, P.J.: The finding of virus-like particles in geometric tubuloreticular structures (GTRS). J Natl Cancer Inst (In Press).

1035. Vogt, P.K.: The Oncovirinae: A definition of the group. In: WHO on Comparative Virology (In Press).

1036. Vogt, P.K.: The genetics of RNA tumor viruses. In Fraenkel-Conrat, H. and Wagner, R. (Ed.): Comprehensive Virology. New York, Academic Press, Inc., 1977, Vol IX (In Press).

1037. Vogt, P.K.: RNA tumor viruses 1975. In Fenoglio, C.M. and King, D.W. (Ed.): Advances in Pathobiology 4: Cancer Biology II, Etiology and Therapy. New York, Stratton Intercontinental Medical Book Corporation, 1976 (In Press).

1038. Vogt, P.K. and Hu, S.S.F.: The genetic structure of RNA tumor viruses. In: Annual Reviews of Genetics. Palo Alto, Ca., Annual Reviews, Inc., 1977, Vol 11 (In Press).

1039. Voyles, B.A. and McGrath, C.M.: Markers to distinguish normal and neoplastic mammary epithelial cells in vitro: Comparison of saturation density, morphology and concanavalin A reactivity. Int J Cancer 18: 498-509, 1976.

1040. Walia, A.S. and Lamon, E.W.: The immune response to polyoma tumor cells in mice. I. The magnitude and specificity of the in vitro cellular cytotoxicity elicited by immunization with graded numbers of tumor cells. J Natl Cancer Inst 1977 (In Press).

1041. Wallen, W.C., Mattson, J.M. and Levine, P.H.: Detection of Epstein-Barr virus soluble antigen by ELISA. J Infect Dis (In Press).

1042. Wallen, W.C., Pearson, G.R., Prevost, J.-M., Neubauer, R.H. and Rabin, H.: Correspondence between T-cell suppression and ADLC titers during Herpesvirus saimiri (HVS) infection in owl monkeys. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

1043. Wang, L.-H., Duesberg, P., Kawai, S. and Hanafusa, H.: Location of envelope-specific and other sequences on the RNA of Schmidt-Ruppin Rous-sarcoma virus. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976, p 542-548.

1044. Wang, L.-H., Duesberg, P., Kawai, S. and Hanafusa, H.: Location of envelope-specific and sarcoma-specific oligonucleotides on RNA of Schmidt-Ruppin Rous sarcoma virus. Proc Natl Acad Sci USA 73(2): 447-451, 1976.

1045. Wang, L.-H., Duesberg, P., Mellon, P. and Vogt, P.K.: Distribution of envelope-specific and sarcoma-specific nucleotide sequences from different parents in the RNAs of avian tumor virus recombinants. Proc Natl Acad Sci USA 73(4): 1073-1077, 1976.

1046. Wang, L.-H., Galehouse, D., Mellon, P., Duesberg, P., Mason, W.S. and Vogt, P.K.: Mapping oligonucleotides of Rous sarcoma virus RNA that segregate with polymerase and group-specific antigen markers in recombinants. Proc Natl Acad Sci USA 73(11): 3952-3956, 1976.

1047. Ward, J.M., Houchens, D.P., Collins, M.J., Jr., Young, D.M. and Reagan, E.L.: Naturally-occurring Sendai virus infection of athymic nude mice. Vet Pathol 12 (In Press).

1048. Waters, R., Bouck, N., di Mayorca, G. and Regan, J.D.: Partial inhibition of postreplication repair and enhanced frequency of chemical transformation in rat cells infected with leukemia virus. Proc Natl Acad Sci USA 74(1): 238-242, 1977.

1049. Wedderburn, N., Carter, R.L. and Salaman, M.H.: Growth of a transplantable lymphoma and its modification in mice infected with the inducing virus. Br J Cancer 34: 390-400, 1976.

1050. Weibel, R.E., Villarejos, V.M., Vella, P.P., Woodhour, A.F., McLean, A.A. and Hilleman, M.R.: Clinical and laboratory investigations of monovalent and combined meningococcal polysaccharide vaccines, groups A and C (39563). Proc Soc Exp Biol Med 153: 436-440, 1976.

1051. Weislow, O.S., Schneider, S., Heberling, R.L., Kalter, S.S. and Hellman, A.: Autogenous humoral immunity to baboon xenotropic endogenous type C virus. J Natl Cancer Inst 57: 561-565, 1976.

1052. Weiss, M., Zeelon, E.P., Sweet, R.W., Harter, D.H. and Spiegelman, S.: Immunological cross-reactions of the major internal protein components from "slow" viruses of sheep. Virology 76: 851-854, 1977.

1053. West, D.L.: Contaminant dispersion and dilution in a ventilated space. ASHRAE Transactions 83(I): 1977 (In Press).

1054. Westmoreland, D. and Rapp, F.: Cell transformation by DNA-containing viruses. In Burger, M.M and Weismann, C. (Ed.): BBA Reviews on Cancer. Amsterdam, The Netherlands, Elsevier Scientific Publishing, 1976, 458, p 167-211.

1055. Westmoreland, D. and Rapp, F.: Host range temperature sensitive mutants of herpes simplex virus type 2. J Virol (In Press).

1056. Westmoreland, D., St. Jeor, S.C. and Rapp, F.: The development by cytomegalovirus-infected cells of binding affinity for normal human immunoglobulin. J Immunol (In Press).

1057. Wettstein, P.J., Krammer, R., Nowinski, R.C., David, C.S., Frelinger, J.A. and Shreffler, D.C.: A cautionary note regarding Ia and H-2 typing of murine lymphoid tumors. Immunogenetics 3: 507-516, 1976.

1058. Williams, R.E., Linsley, P.S., Fox, F., Fittenhouse, E.G. and Boone, C.W.: Lack of constant correlation between normal phenotypic properties and tumorigenicity in variant cell lines. Cell (In Press).

1059. Wilson, C.B. and Dixon, F.J.: Glomerulonephritis: Immunopathology and clinical manifestations. In Samter, M. (Ed.): Immunological Diseases. New York, Little, Brown and Co. (In Press).

1060. Witz, I.P., Lee, N. and Klein, G.: Serologically detectable specific and cross-reactive antigens on the membrane of a polyoma virusinduced murine tumor. Int J Cancer 18: 234-249, 1976.

1061. Wold, W.S.M., Green, M., Brackmann, K.H., Cartas, M.A. and Devine, C.: Genome expression and mRNA maturation at late stages of productive adenovirus type 2 infection. J Virol 20(2): 465-477, 1976.

1062. Wold, W.S.M., Green, M. and Büttner, W.: The Adenoviruses. In Nayak, D.P. (Ed.): Molecular Biology of Animal Viruses. New York, Marcel Dekker, Inc. (In Press).

1063. Wold, W.S.M., Green, M. and Mackey, J.K.: Analysis of human tumors for DNA sequences of the common human DNA tumor virus. In Methods in Cancer Research (In Press).

1064. Wold, W.S.M., Green, M. and Munns, T.W.: Methylation of late adenovirus 2 nuclear and messenger RNA. Biochem Biophys Res Commun (In Press).

1065. Wolfe, L.G. and Deinhardt, F.: Overview of viral oncology studies in saguinus and callithrix species. Primates in Medicine. Basel, S. Karger AG (In Press).

1066. Wong-Staal, F., Gillespie, D.H. and Gallo, R.C.: Proviral sequences of baboon endogenous type C RNA virus in DNA of leukaemic tissues from seven patients with myelogenous leukaemia. Nature (In Press).

1067. Woods, W.A., Papas, T.S. and Chirigos, M.A.: The effect of levamisole on in vitro parameters of the immune response. In Chirigos, M.A. (Ed.): Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasia, Dec 1974. Fogarty Int Ctr Proc No. 28, 1977, p 23-29.

1068. Wright, J., Falk, L.A., Collins, D. and Deinhardt, F.: Mononuclear cell fraction carrying Herpesvirus saimiri in persistently infected squirrel monkeys. J Natl Cancer Inst 57: 959-962, 1976.

1069. Wright, J., Falk, L.A., Wolfe, L.G., Ogden, J.D. and Deinhardt, F.: Susceptibility of common marmosets (Callithrix jacchus) to oncogenic and attenuated strains of Herpesvirus saimiri. J Natl Cancer Inst (In Press).

1070. Wright, P.J., Bernhardt, G., Major, E.O. and Di Mayorca, G.: Comparison of the serology, transforming ability and polypeptide composition of human papovaviruses isolated from urine. J Virol (In Press).

1071. Wright, P.J. and di Mayorca, G.: Radioisotopic labeling of human papovavirus (BK) by iodination and reductive alkylation. J Virol 19: 750-755, 1976.

1072. Wu, A.M.: The biochemical life cycle of type C RNA tumor viruses. In Gallo, R.C. (Ed.): Advances in Cancer Research: Cell Biology, Molecular Biology and Tumor Virology. Cleveland, Ohio, CRC Press, Inc. (In Press).

1073. Wu, A.M. and Gallo, R.C.: Reverse transcriptase. In Fasman, G.D. (Ed.): Critical Reviews in Biochemistry. Cleveland, Ohio, CRC Press, Inc. (In Press).

1074. Wu, A.M. and Gallo, R.C.: The phenotypic abnormality in leukemia: A defective cell-factor interaction? In Neth, R. (Ed.): The Symposium on Modern Trends in Human Leukemia. Wilsede, West Germany, 1975 (In Press).

1075. Wu, A.M., Richardson, L.S., Faran, M. and Gallo, R.C.: A survey on the effect of steroid hormone on type C virus production from cultured murine cells. Cancer Res (In Press).

1076. Wu, A.M., Ruscetti, F.W. and Gallo, R.C.: On the specificity of protein factors affecting growth and differentiation of human hemopoietic cells in culture. Ego Lorenz Memorial Symp (In Press).

1077. Wu, A.M., Schultz, A., Reitz, M.S. and Gallo, R.C.: Posttranscriptional effect of dexamethasone and interferon on the viral replication induced by iododeoxyuridine from murine transformed nonproducer fibroblasts. In Clemmesen, J. and Yohn, D.S. (Ed.): Comparative Leukemia Research 1975, Bibl Haematol, No. 43. Basel, S. Karger AG, 1976 (In Press).

1078. Wu, M., Roberts, R. and Davidson, N.: Structure of the inverted terminal repetition of adenovirus-2 DNA. J Virol 21: 766-777, 1977.

1079. Yajima, Y. and Nonoyama, M.: Mechanisms of infection with Epstein-Barr virus. I. Viral DNA replication and formation of noninfectious virus particles in superinfected Raji cells. J Virol 19(1): 187-194, 1976.

1080. Yajima, Y., Tanaka, A. and Nonoyama, M.: Inhibition productive replication of Epstein-Barr virus DNA by phosphonoacetic acid. Virology (In Press).

1081. Yang, N.S., Soule, H.D. and McGrath, C.M.: Expression of murine mammary tumor virus related antigens in human breast carcinomas (MCF-7) cells. J Natl Cancer Inst 1977 (In Press).

1082. Yefenof, E. and Klein, G.: Difference in antibody induced redistribution of membrane IgM in EBV-genome free and EBV positive human lymphoid cells. Exp Cell Res 99: 175-178, 1976.

1083. Yefenof, E., Klein, G., Ben-Bassat, H. and Lundin, I.: Differences in the Con A induced redistribution and agglutination patterns of EBV genome free and EBV carrying human lymphoma lines. Exp Cell Res (In Press).

1084. Yefenof, E., Klein, G., Jondal, M. and Oldstone, M.B.A.: Surface markers on human B- and T-lymphocytes. IX. Two-color immunofluorescence studies on the association between EBV receptors and complement receptors on the surface of lymphoid cell lines. Int J Cancer 17: 693-700, 1976.

1085. Yefenof, E., Klein, G. and Kvarnung, K.: Relationships between complement activation, complement binding and EBV-absorption by hematopoetic cell lines. Cell Immunol (In Press).

1086. Yefenof, E., Witz, I.P. and Klein, E.: Interaction of antibody and cell surface localized antigen. Int J Cancer 17: 633-639, 1976.

1087. Yeger, H., Kalnins, V.I. and Stephenson, J.R.: Electron microscopy of mammalian type C RNA viruses: Use of conditional lethal mutants in studies of virion maturation and assembly. Virology 74: 459-469, 1976.

1088. Yohn, D.S., Olsen, R.G., Schaller, J.P., Hoover, E.A., Mathes, L.E., Heding, L. and Davis, G.: Experimental oncornavirus vaccines in the feline. Cancer Res 37: 382-387, 1976.

1089. Yoshida, M.C., Saski, M., Takeichi, N. and Boone, C.W.: Karyotypes of vasoformative sarcomas arising from BALB/3T3 cells attached to polycarbonate plates. Cancer Res 36: 2235-2240, 1976.

1090. Young, H.A., Shih, T.Y., Scolnick, E.M. and Parks, W.E.: Steroid induction of mouse mammary tumor virus: Effect upon synthesis and degradation of viral RNA. J Virol 21: 139-146, 1977.

1091. Young, L.J.T., Cardiff, R.D. and McGrath, C.M.: Primary epithelial cell dome culture of mouse mammary tumors. Tissue Culture Association Manual (In Press).

1092. Yount, W.J., Utsinger, P.D., Butt, L.M., Buchanan, E.D., Korn, J., Fuller, C.R., Logue, M. and Pagano, J.S.: Subpopulations of human lymphoblastoid cell lines: Correlation with the expression of surface receptors and content of Epstein-Barr virus genome. Scand J Immunol 5: 795-810, 1976.

1093. Yuhas, J.M., Tennant, R.W. and Regan, J.D. : iology of Radiation Carcinogenesis. New York, Raven Press (In Press).

1094. Yunker, C.E., Clifford, C.M., Thomas, L.A., Keirans, J.E., Casals, J., George, J.E. and Parker, J.C.: Sunday Canyon virus, a new upgrouped agent from the tick Argas (A.) cooleyi in Texas. Archives of Virol (In Press).

1095. Zachrau, R.E., Black, M.M., Dion, A.S., Shore, B., Isac, M., Andrade, A.M. and Williams, C.J.: Prognostically significant protein components of human breast cancer tissues. Cancer Res 36: 3143-3146, 1976.

1096. Zamecnik, P.C.: Protein synthesis - early waves and recent ripples. J Infect Dis (In Press).

1097. Zamecnik, P.C.: Importance of South American non-human primates in human health and medical research. J Infect Dis (In Press).

1098. Zamecnik, P.C.: Protein synthesis - early waves and recent ripples. In Kornberg, A., Horecker, E.L., Cornudella, L. and Oro, J. (Ed.): Reflections on Biochemistry. New York, Pergamon Press, 1976, p 303-308.

1099. Zamecnik, P.C.: Importance of American nonhuman primates in human health and biomedical research. Keynote address. In Monographs of the Pan American Health Organization. 1st Inter-American Conf on Conservation andUtilization of South American Non-Human Primates in Biomedical Research, Lima, Peru, Jun 1975, 1976, p vii-x.

1100. Zamecnik, P.C. and Long, J.C.: Growth of cultured cells from patients with Hodgkin's disease and transplantation into nude mice. Proc Natl Acad Sci USA 74: 754-758, 1977.

1101. Zeve, V.H., Gonda, M.A., Arthur, L.O. and Fine, D.L.: Scanning electron microscopy of an oncornavirus producing mouse mammary tumor cell line, surface localization of virus production during glucocorticoid stimulation. Cancer Res (In Press).

1102. Ziegler, J., Andersson, M., Klein, G., Henle, W.: Detection of Epstein-Barr virus DNA in American Burkitt's lymphoma. Int J Cancer 17: 701-706, 1976.

1103. Ziegler, J.L., Magrath, I.T., Gerber, P. and Levine, P.H.: Epstein-Barr virus and human malignancy. Ann Intern Med 86: 323-336, 1977.

1104. Zinkernagel, R.M. and Dixon, F.J.: Comparison of T cell-mediated immune responsiveness of NZB, (NZB x NZW) F1 hybrid and other murine strains. Clin Exp Immunol (In Press).

1105. Zinkernagel, R.M. and Doherty, P.C.: Does the apparent H-2 compatibility requirement for virus-specific T cell-mediated cytolysis reflect T cell specificity for "altered self" or physiological interaction mechanisms? In The Role of Products of the Histocompatibility Gene Complex in Immune Responses. New York, Academic Press, Inc., 1976, p 203-211.

Aaronson, S.A. 1, 2, 3, 4, 5, 6, 7, 56, 57, 58, 78, 131, 228, 268, 270, 350, 361, 409, 504, 534, 691, 737, 742, 743, 794, 810, 919, 953, 954, 955, 956, 957, 958, 959, 960, 961, 1013
Aaslestad, H.G. 8, 11
Abelev, G.I. 557
Ablashi, D.V. 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 34, 336, 488, 538, 730, 882, 1017
Adam, E. 787
Adams, A. 18
Adams, M.R. 421
Aghai, E. 341
Agrba, V. 272, 273, 767
Ahmed, M. 19, 20, 365
Aisenberg, A.C. 570
Akira, D. 21, 978, 979, 980
Albrecht, T. 22
Aldrich, J.O. 367, 368, 394
Alford, T.C. 609
Al-Ghazzculi, I.K. 23, 737
Ali, M. 52
Allen, P.T. 24
Allen, W.P. 770
Anderson, C. 609
Andersson, B. 191, 192, 547
Andersson, M. 25, 26, 27, 700, 1102
Andersson-Anvret, M. 28, 341
Andrade, A.M. 1095
Aoki, T. 29, 30, 31, 32, 33, 117
Arcement, L.J. 642
Arens, M. 459
Arlinghaus, R.B. 462, 463, 642, 813, 1020
Armstrong, G.E. 9, 10, 12, 16, 17, 34, 1017
Arndt, A. 802
Arnold, T. 431, 432
Arnstein, P. 35, 650, 652, 785, 798
Arthur, L.C. 36, 283, 1101
Arthur, M. 585
Ash, J.F. 37
Asher, Y. 65, 82, 896, 898
Ashley, R.L. 38, 140
Ashman, R. 528, 639, 696
Åsjö, B. 308, 506
Atine, I. 700
Au, W. 604
August, J.T. 39, 88, 457, 537, 557, 614, 883, 884, 885, 904, 905, 913, 963, 964, 965, 966, 967, 968
Aulakh, G. 362
Auletta, A.E. 757
Aurelian, L. 40, 41, 42, 43, 44, 45, 461, 970
Austin, F. 46, 680
Avila, J. 47
Axel, R. 48

Baggs, R. 194, 211
Bailey, J.M. 539
Bakacs, T. 1021
Baker, L.E. 344
Baldi, A. 806
Baldwin, C.I. 49
Balfour, H.H. 430, 433
Baltimore, D. 50, 51, 366, 370, 540, 606, 736, 820, 906
Baluda, M.A. 52
Bandyopadhyay, A.K. 36
Bank, A. 553, 673, 674, 689
Baptista, J. 394
Barahona, H. 53, 54, 55, 193, 511
Barbacid, M. 56, 57, 58, 534, 957
Barker, S.T. 379, 920
Barker, S.K. 818
Barnes, D.M. 729
Barnes, R.D. 59
Baron, J. 667
Baroudy, E.M. 60
Barr, R.D. 366
Bassin, R.H. 254, 499, 790, 791
Baumgarterer, L. 134
Beard, D. 61
Beard, J.W. 61
Becker, L.E. 62
Becker, S. 63, 64, 515
Becker, Y. 65, 82, 83, 295, 296, 481, 699, 896, 897, 898
Bedigian, H.G. 996
Beemon, K. 66, 67, 229, 250
Bekesi, J.G. 53, 68, 69, 70, 71, 72, 190, 424, 511, 542, 814, 815, 850
Bell, C. 932, 933, 934
Bell, R.E. 44
Bellomy, E.E. 1026
Ben-Bassat, H. 73, 74, 341, 1083
Bendas, C.M. 75
Bender, J. 550
Bender, W. 76, 249, 539
Benedict, D. 836
Benedict, W.F. 291
Benjamin, T. 844
Benjamin, T.L. 77, 281, 286, 948
Bensinger, W.I. 78, 350
Benton, E. 182, 259, 260, 367, 368, 394, 745
Benton, C.V. 79, 471
Bentvelzer, P. 80, 408, 666
Bentwich, Z. 73
Benveniste, R.E. 81, 136, 892, 893, 1010, 1012
Benyesh-Melnick, M. 267, 276
Ben-Zeev, A. 82, 83, 481
Berek, C. 84
Bergh, O.J. 246
Bergholz, C. 85, 86, 272, 273
Berman, P.E. 550
Bernhardt, G. 588, 630, 1070
Bernstein, I. 87
Bigner, D.D. 171, 172
Bilello, J. 88
Billiau, P. 886
Billing, P.J. 246, 523, 524
Bishop, D.E.I. 170

Bishop, J.M. 89, 181, 297, 804, 949
Bister, K. 90
Black, M.M. 91, 92, 93, 94, 95, 96, 1095
Black, P.H. 97, 98, 145, 415, 416, 503, 761, 762, 803
Blair, D.G. 99
Blair, P.E. 654, 804
Blake, J.E. 447
Blakemore, W.S. 912
Blakeslee, J.F. 381, 847
Blank, K. 100, 101
Blattner, W.A. 498, 577, 578, 579
Bloomer, J.R. 102
Bobrove, A.M. 299
Boehm-Truitt, M. 859
Boirón, M. 995
Bokish, V. 477
Bolio, L.D. 179
Bolognesi, D.P. 103, 171, 172, 179, 211, 423, 454, 455, 456, 458, 541, 631, 749, 838, 839, 840, 841
Bonavida, B. 506
Bondurant, M. 104
Bone, D.R. 842
Bonkowsky, H.L. 102
Bonnard, G.D. 105
Boobis, A.R. 106, 643, 644
Boone, C.W. 46, 107, 108, 109, 110, 111, 258, 332, 679, 680, 683, 723, 984, 985, 986, 987, 988, 989, 990, 1058, 1089
Borken, S. 430
Bosch, V. 541
Bouck, N. 665, 1048
Bowen, J.M. 112, 113, 237, 238, 690
Boyars, J.K. 1014
Boyd, A.L. 114, 115
Boyse, E.A. 116
Brackmann, K.H. 1061
Bradley, R. 824
Brandchaft, P.B. 31, 117
Brennan, P.J. 118
Breslow, R. 793
Breznock, A.W. 119, 501
Brinkman, E.J. 811
Britton, S. 816
Broseus, I. 769
Brown, A. 1001
Brown, D. 508, 509
Brown, J.C. 314, 319, 322
Brown, J.M. 120
Brown, J.P. 121
Brown, M. 280
Brown, R. 453
Brown, S.E. 585
Brown, T.M. 122
Brugère, J. 220
Bryant, M.L. 123, 262
Bubbers, J.E. 124
Buchanan, P.D. 1092
Buehring, G.C. 125, 801
Buell, D.N. 256, 257
Buell, P. 126, 396, 574, 575
Burke, M.D. 429
Burke, S. 451
Burstein, S.R. 127
Burstein, Y. 848
Bushar, G.S. 31, 32, 33
Buss, E.F. 776
Büttner, W. 128, 1062
Buynak, E.B. 129
Byers, V.S. 130

Cabradilla, C. 5, 131, 810, 958
Cabral, G.A. 132
Calafat, J. 133
Caldwell, G.G. 134, 135, 564
Callahan, R. 81, 136, 137
Cameron, J.R. 713
Cammoun, N. 220
Campbell, D.A. 105
Canaani, E. 138
Cannon, G.B. 200, 201, 609
Cantor, H. 116, 906
Cardiff, R.D. 38, 139, 140, 654, 805, 1003, 1091
Carlson, J.V. 141
Cartas, M.A. 1061
Carter, C. 134
Carter, R.L. 1049
Casagrande, J.C. 142, 317, 318, 395, 397, 584
Casals, J. 1094
Casey, J. 143
Cedar, H. 144
Cerny, J. 145
Chakrabarty, M. 361
Chalopin, J.M. 543
Chan, E. 146, 784
Chan, J.C. 238, 255
Chan, S.H. 147, 148, 149, 150, 151, 152, 908, 910
Chander, S. 153, 227
Chang, E.H. 294
Chang, S.-Y. 363
Chaparas, S.D. 164
Chapman, V. 81
Charman, H.P. 154, 155, 314, 321, 705, 821
Charnay, N. 328
Charney, J. 632
Chatigny, M.A. 156
Chau, J.C.W. 418, 420, 908, 911
Cheeseman, S.H. 157
Chen, H.W. 158, 159
Chen, Y.C. 160
Chermann, J.-C. 17
Chew, T.S. 147, 148, 149, 150
Chi, J. 485, 693
Chinnadurai, G. 161, 162, 348
Chirigos, M.A. 163, 164, 165, 627, 628, 629, 715, 716, 759, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 1067
Chirikjian, J.G. 60, 166, 716, 717
Cho, H.Y. 167, 796, 797

Cho, J.-R. 189
Chopra, H.C. 168
Chung, K.C. 755
Chuvirov, G.N. 767
Cicmanec, J.L. 767, 768
Claviez, M. 839
Clements, G. 169, 519, 520, 523, 524
Clewley, J.P. 170
Clifford, C.M. 1094
Cloyd, M.W. 171, 172
Coates, H. 730
Cochrane, C.G. 173
Cockerell, G.L. 174, 175, 176, 427, 535, 536
Coffin, J. 170, 177
Coggin, J.H. 1026
Cohen, I.R. 901
Cohen, M.M. 73
Cohen, S. 1011
Cohen, Y. 65
Colcher, D. 178, 247, 248, 434, 851, 852, 853, 854, 855, 857
Cole, G.A. 461
Collins, D. 1068
Collins, J.J. 179, 454, 455, 456, 840
Collins, M.J. 105, 1047
Conlon, T. 932, 933
Connan, G. 180
Connelly, R. 15, 406, 564
Connor, R.J. 609
Cooper, N.R. 465
Cordell, B. 181
Cornain, S. 1021
Cornish, J.D. 43
Cosgrove, M. 182, 394
Costa, J. 293, 580
Cotter, S. 134, 268, 270
Courtney, R.J. 118, 183, 184, 232, 285, 750, 842, 843
Cox, B. 292
Creemers, P. 80, 185
Crittenden, L.B. 186, 187, 188, 919
Croce, C. 966, 967
Croce, C.M. 507
Croker, B.P. 206, 233, 234
Crowell, R.L. 75
Cuatico, W. 189
Currier, R. 134
Cutchins, E.C. 167
Cuttner, J. 190, 424

Dahlberg, J.E. 616, 736, 856
Dalianis, T. 191, 192
Dalton, A.J. 16
Daly, T.M. 888, 889, 890
Daniel, M.D. 53, 54, 55, 193, 194, 447, 511
Darai, G. 637
Das, M.R. 195
David, C.S. 1057
Davidson, N. 76, 143, 249, 438, 439, 480, 539, 603, 1078
Davis, D.S. 436
Davis, G. 1088
Davis, J. 196, 197
Davis, R.W. 713
Davoli, I. 198
Day, N.E. 199, 221, 328, 908, 909, 910, 911
Dean, J.B. 200, 201, 735
Deinhardt, F. 85, 86, 202, 203, 204, 272, 273, 274, 275, 276, 610, 637, 684, 1065, 1068, 1069
DeJak, D. 688
Delarco, J. 205, 271, 1011
Delatto, F. 485
Del Guidice, R. 375
Delotto, R. 819
Del Villano, B.C. 206, 207, 208, 209, 210, 233, 694
Denny, T.P. 179, 749
De Noronha, F. 211, 839
Depue, R. 660
Derge, J.G. 361, 362, 363
Descalzi, C.C. 421
Desgranges, C. 212, 213, 214, 877
De Thé, G. 150, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 274, 328, 337, 339, 543, 544, 545, 559, 641, 877, 908, 910, 911
Devare, S.G. 153, 227, 228, 754
Devine, C. 1061
Dharmalingam, S. 911
Didier-Fichet, M.L. 16, 641
Diegmann, F. 314
di Mayorca, G. 284, 588, 589, 630, 665, 822, 1048, 1070, 1071
Dina, D. 138, 229, 587
Dion, A.S. 95, 230, 231, 833, 889, 1095
Diwan, B.A. 288
Dix, R.D. 232
Dixon, F.J. 173, 206, 233, 234, 466, 467, 694, 695, 1059, 1104
Dmochowski, L. 112, 113, 235, 236, 237, 238, 239, 255, 410, 599, 690, 760, 878
Dodson, M.L. 112, 238
Doherty, P.C. 1105
Dölken, G. 240, 241
Dollbaum, F.M. 923
Doller, E.W. 242, 243
Donahoe, R.M. 23, 504, 737, 961
Donnelly, J. 1016
Dorfman, R.A. 557
Dorfman, R.F. 264
Dorizzi, M. 244
Dosik, H. 245, 577, 1028, 1029, 1030, 1031, 1032
Dougherty, M. 315, 317, 318, 821
Doyle, T. 668, 669, 670
Dray, S. 630
Drew, S.I. 246
Drohan, W. 178, 247, 248, 851, 852, 853, 854, 855, 857

Drohan, W.N. 52
Dube, S. 249
Dubouch, P. 214
Duesberg, P. 66, 138, 229, 250, 251, 306, 307, 587, 1043, 1044, 1045, 1046
Dumont, J. 252
Dunn, C.Y. 361
Dupont, E. 253
Duran-Troise, G. 254, 790, 791
Dworsky, R.L. 314, 322, 364

East, J.L. 255
Easton, J.M. 8, 9, 10, 11, 12, 13, 14, 15, 17, 336, 730, 1017
Ebbesen, P. 406
Eberle, R. 277
Ebert, P.S. 102, 256, 257, 907
Economou, C.K. 258, 987, 988
Edmondson, H.A. 259, 260, 745
Ehlin, M. 1029
Eichberg, J.W. 261
Eichmann, K. 84
Elder, J.H. 209, 262, 263
Elliot, A. 847
Ellis, D.A. 762
Ellison, R.R. 424
Ellouz, R. 213, 220
Emery, S. 555
Enright, F.M. 770
Epstein, A.L. 264
Epstein, P.S. 244, 265
Ernberg, I. 266, 326
Espana, C. 488
Esparza, J. 267
Essex, M. 134, 155, 268, 269, 270, 600, 918, 959
Estes, J.D. 315, 316, 317, 318, 320, 526

Fabricant, R.N. 271
Fabrizio, P. 564
Falk, L.A. 203, 272, 273, 274, 275, 276, 1068, 1069
Faras, A.J. 66
Farber, F.E. 277
Fareed, G.C. 198
Farrelly, J.G. 479
Faulkin, L.J. 139
Favre, M.C. 559
Feingold, N. 252
Fellows, C. 10
Felsburg, P.J. 491
Fenyö, E.M. 63, 278, 279
Ferdinand, F.-J. 280
Ferrone, S. 339
Feunteun, J. 281
Fibach, E. 282, 593
Fields, B.N. 788, 976
Fine, D.L. 36, 95, 283, 760, 1101
Fiori, M. 284, 589
Firtel, R.A. 915
Fischer, I. 538, 882
Fischinger, P.J. 289, 290, 454, 455, 456, 661, 662, 813, 839, 840, 1020
Fish, D.C. 446
Fisher, I. 952
Flandermeyer, R.R. 649
Flannery, V.L. 285
Flax, R.L. 302, 303
Fleckenstein, B. 636
Fluck, M.M. 281, 286, 948
Forbes, M. 193
Forsby, N. 28
Fowler, A.K. 24, 287, 385, 386, 533, 969
Fox, F. 1058
Fox, R.R. 288
Frank, H. 103, 839
Frankel, A.E. 289, 290
Fraser, C.E.O. 53
Fraumeni, J.F. 498, 577, 578, 579
Freedman, E. 100
Freeman, A.E. 291, 783
Frelinger, J.A. 1057
Frenkel, N. 292
Fried, J. 1002
Friedman, E.M. 293, 294
Friedmann, A. 295, 296, 897, 898
Friedrich, R. 181, 297
Friend, K. 298
Friesen, L. 836
Frost, E. 120
Fuhrman, E.S. 629
Fujita, D. 89
Fuks, Z. 299, 300, 301
Fuller, C.R. 1092
Funderburk, W.W. 302, 303
Furmanski, P. 304, 611, 800
Fuson, E.W. 305, 549

Gabbiani, G. 544, 545
Gacto, M. 917
Galehouse, D. 306, 307, 1046
Galili, N. 308, 506
Gallagher, R.E. 146, 309, 310, 312, 691, 792
Gallo, R.C. 146, 309, 310, 311, 312, 633, 691, 792, 811, 1066, 1073, 1074, 1075, 1076, 1077
Ganem, D. 198
Gardner, M.B. 154, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 359, 364, 396, 525, 526, 603, 604, 657, 658, 783, 784, 785, 786, 821
Garrett, E.W. 960
Gartner, S. 494
Gaudrau, F. 952
Geder, L. 323, 324, 325, 338
Geltosky, J.E. 744
George, J.F. 1094
Gerard, G.F. 592
Gerber, P. 1103
Gergely, P. 326, 816

Gerkins, V.E. 142, 395, 397, 585
Gernand, L. 291
Gershwin, M.E. 327
Gerwin, B.I. 127, 254, 499, 731, 790, 791
Geser, A. 221, 222, 223, 328
Ghanta, V. 547
Gheridian, B. 762
Giard, D.J. 498
Gilbert, W. 371
Gilden, R.V. 111, 154, 155, 196, 314, 316, 321, 329, 443, 444, 445, 446, 486, 526, 603, 655, 691, 692, 758, 769, 796
Gilead, Z. 161, 330, 331, 464, 973, 974
Gillespie, D.H. 309, 310, 312, 792, 1066
Gillette, R.W. 332
Ginsburg, C.M. 333, 334
Giovanella, B.C. 226, 656
Gisselbrecht, S. 499
Glaser, R. 13, 335, 336, 337, 338, 339, 619, 663
Glidewell, C. 424
Goh, E.H. 910
Goldberg, R.J. 75, 340, 725, 873, 874
Goldblum, N. 73, 74, 341
Goldblum, T. 73, 341
Goldstein, G. 906
Golub, M.M. 1013
Gonda, M.A. 1101
Goodman, H.M. 181, 297, 560
Goodman, M.L. 342
Goodson, S. 549
Gordon, M. 398
Gordon, R.J. 343
Gottlieb, J.A. 344
Gottlieb, P.D. 540
Graham, B. 932, 933
Graham, B.J. 812, 907
Gravell, M. 340, 345
Green, I. 797, 799, 1033
Green, M. 104, 128, 161, 162, 330, 331, 346, 347, 348, 349, 369, 372, 459, 464, 586, 592, 710, 832, 973, 974, 1061, 1062, 1063, 1064
Green, M.E. 348, 349
Green, R.W. 458, 839
Greenber, J.S. 78
Greenberger, J.S. 350, 743
Greenlee, J.E. 351, 470
Groff, D.E. 466, 467
Gross, L. 352
Guegan, J.H. 14
Gunnell, M. 498
Gunvén, P. 353
Gupta, P. 354, 355, 356, 357
Gustafsson, L.A. 253

Haaijman, J.J. 358
Haapala, D.K. 813, 1020
Haase, A.T. 66
Hackett, P.J. 130, 359, 360, 923, 942, 943, 944
Hageman, P.C. 133
Haguenau, F. 338
Halden, E.T. 985
Hale, F. 548
Hampar, B. 361, 362, 363, 769, 994
Hampton, J. 263
Hanafusa, E. 250, 1043, 1044
Hanisch, R. 364
Hanna, M.G. 454, 455, 456
Hanson, C.A. 794
Harada, F. 736
Hardistry, R.M. 928
Hardy, W.D. 134, 155, 268, 270, 426, 427, 559
Harewood, K.R. 19, 365
Hariri, F. 655
Harris, C.L. 812
Harris, R. 932, 933, 934
Harrison, T.A. 51, 366, 606
Harrold, J.E. 119, 501
Hart, W.R. 367, 368, 394
Harter, D.F. 1052
Harter, M.L. 330, 369
Hartley, J.W. 30, 662
Harvey, J.W. 491
Haseltine, W. 177
Haseltine, W.A. 370, 371, 736
Hashimoto, S.-I. 372
Hashimoto, S.I. 104
Hatanaka, M. 362, 373, 374, 375, 376, 486, 691, 692
Hatgi, J. 471
Hatheway, J.M. 912
Hauptfeld, M. 525
Hauser, D.E. 314
Hay, K.A. 553
Hayman, E.G. 377
Hayman, M.J. 160, 378, 448
Hays, E.F. 670
Heasley, S. 1024
Heath, C. 564
Heath, C.W. 122, 135
Heberling, R.L. 321, 379, 488, 489, 490, 491, 920, 921, 1051
Heding, L. 380, 381, 697, 1088
Hehlmann, R. 382
Heimer, F. 383
Heine, U. 16, 230, 869, 870
Heiniger, E.J. 158, 159, 384
Hellman, A. 24, 287, 385, 386, 533, 969, 1051
Hellström, I. 87, 298, 387, 388, 389, 390, 391, 392, 393, 487, 651, 991
Hellström, K.E. 87, 121, 298, 387, 388, 389, 390, 391, 392, 393, 487, 645, 646, 651, 991
Helmke, F.J. 920, 921
Henderson, E. 220

Henderson, B.E. 126, 142, 182, 259, 260, 317, 318, 319, 321, 322, 364, 367, 368, 394, 395, 396, 397, 468, 469, 500, 526, 574, 575, 583, 585, 615, 745, 746
Henderson, G.B. 398
Hendricks, M.I. 1005
Henke, C.B. 49
Henle, G. 272, 273, 275, 333, 334, 399, 402, 403, 404, 405, 429, 430, 431, 433, 660
Henle, W. 25, 27, 28, 126, 252, 272, 273, 275, 333, 334, 336, 400, 401, 402, 403, 404, 405, 429, 430, 431, 432, 433, 550, 660, 700, 1102
Heppner, G.H. 542
Herberman, R. 660, 735
Herberman, R.B. 30, 105, 200, 201, 609, 985
Herman, A.C. 631
Herman, M. 264
Herndon, R.M. 351
Herzenberg, L. 739
Hesse, J. 406
Heston, W.E. 407
Heubel, G. 1018
Hewetson, J.F. 550
Hierholzer, J.C. 503
Hildebrand, D.F. 421
Hilgers, J. 59, 133, 214, 274, 408, 1022
Hill, P.E. 443, 444, 445, 446
Hilleman, M.R. 129, 1050
Hiller, A.J. 922
Hino, S. 5, 6, 409, 737, 957, 959, 960, 961
Hiraki, S. 410
Hirsch, M.S. 157, 411, 412, 413, 414, 415, 416, 503, 761, 762, 803
Hixson, D.C. 598
Ho, J.H.C. 213, 220, 221, 224, 328, 337, 417, 418, 419, 420, 440, 543, 908, 911
Hoagland, C. 424
Hodgen, G.D. 421
Holben, J.A. 422, 632
Holden, H.E. 20
Holder, W.D. 423
Holland, J.F. 68, 69, 70, 71, 72, 190, 424, 814
Holliday, J. 59
Hong-Goh, E. 149
Honig, R. 523, 524
Hoogstraten, B. 344
Hoover, E.A. 174, 175, 176, 380, 425, 426, 427, 428, 600, 692, 697, 698, 845, 846, 1088
Hopkins, F.F.I. 652
Horecker, B.L. 913
Horoszewicz, J.S. 239
Horton, R.E. 162
Horwitz, C.A. 334, 429, 430, 431, 432, 433
Houchens, D.P. 1047
Howard, D.K. 434
Howard, E. 314
Howard, T. 569
Howk, R.S. 732
Howley, P. 198, 435, 508, 509
Hsu, H.W. 436
Hsu, I.C. 437
Hsu, K.C. 32, 33
Hu, R. 688
Hu, S. 438, 439, 480, 539
Hu, S.S.F. 1038
Huang, C.-E. 712
Huang, D.P. 440
Huang, E.-S. 441
Huang, K.-Y. 23
Huang, Y.-T. 712
Hubbell, E.S. 725
Huebner, R.J. 35, 167, 314, 315, 316, 317, 318, 319, 320, 321, 384, 442, 443, 444, 445, 446, 556, 757, 758, 759, 783, 796, 798
Huennekens, F.M. 398
Hugunin, P.M. 757
Hull, R.N. 488
Hunsman, G. 839
Hunt, R.D. 53, 55, 193, 194, 447, 815
Hunter, E. 90, 99, 378, 448, 449, 1027
Hunter, I. 450
Hurst, M.M. 547
Hutt, L.M. 1092
Hutt, R. 777, 946
Hyman, R.W. 451, 452, 675, 728, 778

Ibrahim, A. 453
Igel, H.G. 291
Igel, H.J. 724
Ihle, J.N. 454, 455, 456, 505, 526, 556
Ikeda, E. 484
Ikeda, R.M. 327
Iltis, J. 778
Ilyin, K.V. 760
Imamura, E. 457
Ingalls, J.K. 53, 54
Isaacs, E. 604
Isac, M. 1095
Isenberg, W. 828
Ishizaki, R. 458
Israel, E. 508, 509
Ito, K. 459
Iwai, H. 460

Jackman, D. 194
Jacobs, J.E. 108
Jacobs, R.P. 44, 461
Jaenisch, R. 968
Jamjoom, G.A. 462, 463, 642
Jarrett, C. 268
Jasmin, C. 17
Jay, F.T. 294

Jeng, Y.-H. 161, 330, 464
Jensen, F.C. 233, 234, 262, 465
Jenson, A.B. 466, 467
Jenssen, H.L. 727
Jerina, D.M. 643, 644
Jerome, L.F. 609
Jimenez, H. 635
Jing, J. 396, 468, 469, 574, 575
Johansson, B. 74
Johnson, D. 274
Johnson, D.E. 599
Johnson, D.L. 501
Johnson, K.H. 729
Johnson, R.T. 62, 351, 470
Johnson, R.W. 79, 471
Johnston, J.O. 130, 922
Jondal, M. 472, 473, 474, 475, 476, 477, 478, 515, 523, 524, 816, 1084
Jones, T.C. 53, 511
Jones, W.I. 79
Joseph, D.R. 479
Junghans, R.P. 480

Kaaden, C.R. 481
Kacian, D.L. 482, 483
Kaddu-Mukasa, A. 700
Kahn, F. 245
Kai, K. 484
Kaji, A. 485, 693, 819
Kakefuda, T. 486
Kall, M.A. 487
Kalnins, V.I. 1087
Kalter, S.S. 261, 379, 488, 489, 490, 491, 920, 921, 1051
Kandutsch, A.A. 158, 159
Kang, C.-Y. 170, 492
Kaplan, E.J. 368
Kaplan, H.S. 264, 299, 300, 301, 493, 494
Kaplan, J. 120, 495, 496, 497
Kaplan, M.M. 498
Karshin, W.L. 642
Kashmiri, S.V.S. 499, 790, 791
Katz, A.D. 500
Katz, S.L. 54
Kawai, S. 250, 1043, 1044
Kawakami, T.G. 119, 327, 501
Kay, H.D. 502, 914
Keirans, J.E. 1094
Keith, J.M. 67
Keller, E.W. 503
Kelloff, G.J. 23, 504, 737, 837, 961
Kemeny, E.A. 779
Kende, M. 737
Kennel, S.J. 207, 209
Kenney, F.T. 479, 505
Kettmann, R. 248, 851, 852
Keydar, E. 506
Khiroya, R. 1016
Khoury, G. 47, 198, 280, 435, 507, 508, 509, 510, 597, 894
Kiessling, R. 739
Kim, H. 264
Kim, J.M. 797
Kim, N. 154, 155
Kimball, P. 853, 854, 857
King, D.F. 301
King, M.F. 757
King, N.W. 53, 54, 511
Kisuule, A. 634
Kiuchi, M. 512
Klecker, J. 932, 933, 934
Kleid, D.G. 370
Klein, D.L. 513
Klein, E. 63, 64, 73, 246, 278, 478, 514, 515, 521, 522, 986, 1021, 1086
Klein, G. 25, 27, 28, 73, 74, 169, 191, 192, 220, 226, 240, 241, 279, 308, 341, 383, 477, 478, 506, 516, 517, 518, 519, 520, 521, 522, 523, 524, 581, 625, 626, 656, 686, 700, 739, 816, 975, 1060, 1082, 1083, 1084, 1085, 1102
Klein, J. 525
Kleiner, G. 681
Klement, V. 123, 315, 316, 317, 318, 319, 320, 321, 322, 526, 527, 821
Klitzman, L. 121
Knesek, J.E. 255
Kniazeff, A.J. 650
Knight, C.A. 480
Kniker, W.T. 491
Knipscher, R.C. 1033
Kobayashi, H. 989
Kodama, T. 989
Kohl, S. 528, 696
Köhler, E. 727
Koide, Y. 529, 980
Kong, Y.P. 120
Koo, G.C. 245
Korb, J. 230
Korn, J. 1092
Korol, W. 20
Kosky, P. 195
Kouri, P.E. 106, 530, 531, 532, 643, 644
Kourilsky, F.M. 252
Kouttab, N.M. 267, 386, 533, 969
Krakower, J.M. 534
Krakowka, S.G. 174, 175, 176, 535, 536
Kramarsky, B. 934
Krammer, R. 1057
Krantz, M. 537
Kreider, J. 324
Krempin, H.M. 655
Kreutner, A. 200
Krontiris, T.G. 929
Krueger, G.R. 15, 538, 882
Kudler, I. 451, 452
Kufe, D.W. 146
Kuichi, M. 981
Kumar, M.C. 729
Kung, H.J. 249, 539
Kung, P.C. 51, 540, 606

Kurth, R. 541
Kvarnung, K. 1085
Kwan, H.C. 419, 420

Lai, C.-J. 508, 509
Lai, M. 635
Lai, P. 203
Laing, C.A. 542
Lake, R.S. 291
Lambright, P. 492
Lamelin, J.P. 543, 544, 545
Lamellin, J.P. 565
Lamon, E.W. 269, 305, 546, 547, 548, 549, 1040
Lampert, P.W. 695
Land, V.J. 345
Lane, P.E. 743
Lane, S.E. 505
Lane, W.T. 443, 444
Lang, R.M. 122
Lange, B.J. 550
Langlois, A.J. 839
Lapin, B. 272, 273
Lapin, B.A. 203, 767
Larsen, C.J. 995
Larson, L. 304
Lasfargues, E.Y. 551, 552, 1018
Lasfargues, J.C. 552, 1018
Latham-Griffin, E. 901
Lausch, R.N. 553, 554, 638, 752
Lavelle, G.C. 1000
Lavoué, M.F. 220, 877
LeCam, L. 130
Ledbetter, J. 555
Lee, J.C. 454, 455, 456, 556
Lee, K.-L. 505
Lee, L. 362
Lee, N. 1060
Lee, S. 695
Lee, S.K. 122
Lehman, I.R. 713
Leibold, W. 709
Leis, H.P. 95
Leiseca, S.A. 596
Lejneva, O.M. 557
Lemons, R. 558
Lennette, E.H. 35, 488
Lenoir, G. 214, 225, 337, 339, 363, 559
Leonard, C.M. 200
Leong, J.C. 560, 925
Lerner, R.A. 207, 208, 209, 210, 233, 262, 263, 744, 915, 916
Levin, A.G. 659
Levin, A.S. 130
Levin, R. 561
Levine, P.H. 15, 149, 151, 152, 342, 345, 406, 418, 562, 563, 564, 565, 730, 735, 1019, 1041, 1103
Levy, B. 768
Levy, J.A. 566
Lewis, A.M. 724
Lewis, F.B. 431, 432
Li, J.-I.E. 887
Li, J.H. 567, 780
Liabeuf, P. 252
Lidin, E. 549
Lieber, E.M. 568, 893
Lightbody, J.J. 120
Lilly, F. 100, 101, 124
Lindahl, T. 26, 686, 700
Linkhart, S. 670
Linn, J. 1024
Linsley, P.S. 1058
Liu, M. 30, 31, 32, 33
Livingstor, D.M. 569
Locker, H. 292
Loeb, W.F. 34, 652
Loeckner, C.P. 304
Log, T. 834
Logue, M. 1092
Long, C. 197, 375, 376, 701, 702, 769
Long, J.C. 570, 1100
Long, L.K. 961
Longley, C. 304
Loomis, W.F. 915, 916
Lopez, D.M. 244, 265, 571, 572, 573, 902, 903
Louie, E. 126, 396, 468, 469, 574, 575
Lovinger, G.G. 486
Lubiniecki, A.S. 498, 576, 577, 578, 579, 580
Lubs, H.A. 1030
Luetzeler, J. 17, 867
Lui, M. 440
Luka, J. 581, 686
Lund, J.K. 1003
Lundin, I. 1083
Lyons, I.E. 590

MacIntyre, R.J. 582, 682
Mack, T.M. 142, 394, 583, 584, 585
Mackey, J.K. 346, 348, 349, 586, 1063
Madden, M.J. 812
Madin, S.E. 450
Magrath, I.T. 1103
Mahoney, M.J. 102
Maisel, J.E. 66, 587
Major, E.C. 588, 589, 630, 1070
Malherbe, H.H. 488, 490
Malone, J.M. 291
Manders, E.K. 105
Manning, J.S. 38
Manousos, M. 19
Marciani, D.J. 590, 591, 717, 718
Marck, C. 291
Marczynska, B. 86, 637
Margalith, M. 592
Marineau, E.J. 612
Markham, F.D. 52
Marks, P.A. 282, 593, 673, 674, 689, 793, 1002
Márquez, I.D. 594, 595

Martin, D.F. 596
Martin, D.S. 807, 808
Martin, J.W. 720
Martin, L.N. 770
Martin, M.A. 198, 435, 597
Martin, R.G. 47
Martin-Berthelon, M.C. 559
Maruyama, K. 112, 255, 598, 599
Maryak, J. 874
Mason, T. 498
Mason, W.S. 99, 251, 1046
Mathes, L.F. 425, 426, 427, 428, 600, 601, 697, 698, 1088
Mattern, C.F.T. 662
Mattson, J.M. 1041
Maurer, F.H. 525
Maxam, A. 371
May, D. 602
May, E. 510
Mayyasi, S.A. 19, 20, 952
McAllister, R.M. 154, 321, 438, 539, 603, 604, 605, 655, 705, 798, 912
McBride, C.M. 113
McCaffrey, R.P. 51, 366, 606
McClung, H. 607, 608
McConahey, P. 315, 320
McConahey, P.J. 206, 234, 466, 467
McConnell, S. 488
McCormick, J.J. 611, 800
McCoy, J.I. 200, 201, 609, 735
McCullough, B. 261
McDonald, R. 610
McDowell, T.S. 501
McGaw, J. 453
McGrath, C.M. 611, 612, 613, 800, 824, 825, 826, 1039, 1081, 1091
McIntyre, R.F. 345
McLean, A.A. 129, 1050
McLellan, W.I. 614
McMichael, A. 299
Medeiros, E. 670
Meier, H. 288, 384, 996, 997
Melendez, L.V. 54, 55, 193, 194
Melewicz, F. 899
Mellon, P. 251, 1045, 1046
Mellors, R.C. 457
Menck, H.R. 126, 615
Merryman, C.F. 525
Meyer, C. 918
Michalides, R. 616, 617, 852, 853, 854, 856, 857
Michelson-Fiske, S. 338
Mickey, M.R. 564, 979, 982
Mikulski, S.M. 253
Milgrom, H. 899, 900
Milleck, J. 618
Miller, D. 342
Miller, E.C. 671
Miller, M.F. 112, 113, 238, 598
Miller, N.R. 310, 312, 792
Miller, O.J. 689
Miller, R.L. 602, 619, 620, 621
Milo, G.E. 847
Mims, S.J. 294
Minowada, J. 246
Mirkovic, R. 750
Mishra, N.K. 622, 623
Mitrani, S. 73, 74, 341
Miyagi, E. 993
Moar, M. 624, 625, 626
Moennig, V. 454, 455, 456, 839
Mohr, S.J. 164, 627, 628, 629, 862, 871
Molinaro, G.A. 630
Monguet, D. 641
Montelaro, R.C. 103, 631
Moore, D.H. 231, 422, 552, 632, 1018
Mordhorst, C.H. 406
More, N.S. 1033
Morgan, I. 633
Morris, P. 836
Morris, V.L. 297
Morrow, F.H. 634
Moscovici, C. 635
Moscovici, M. 635
Motta, J.V. 187
Moulds, J. 433
Muenz, L. 220
Muir, C. 224
Mulcahy, F.F. 410
Mulder, C. 636
Mullen, Y. 978
Mullins, J.A. 24
Munk, K. 637
Munns, T.W. 1064
Munoz, N. 223
Murasko, D.M. 553, 638
Muto, M. 485, 693
Myer, F.E. 1001
Myers, E. 238
Myers, J.C. 482, 483
Myers, M.W. 294

Nachtigal, M. 22
Nahmias, A. 453, 528, 639, 640, 696, 899, 900
Naor, D.A. 308, 506
Narayan, C. 62, 351, 470
Nardon, E. 641
Naso, R.B. 462, 463, 642, 813, 1020
Natarajan, A.T. 624
Nave, B. 210
Nebert, D.W. 106, 532, 643, 644
Neel, E. 730
Nelson, K. 645, 646
Nelson-Rees, W.A. 359, 568, 647, 648, 649, 650, 707, 708, 785, 870, 922, 923, 942, 943
Nepom, J.T. 651, 991
Neubauer, R.H. 652, 653, 767, 768, 1042
Newgard, K.W. 654
Ng, M.H. 419, 420, 440
Nicolson, E.O. 438, 527, 539, 603, 605, 655
Nilsonne, U. 1021
Nilsson, K. 656

Niman, H.I. 657, 658
Nishioka, K. 659
Nkrumah, F. 660
Nomura, S. 661, 662
Nonoyama, M. 203, 272, 273, 336, 363, 637, 663, 664, 993, 994, 1079, 1080
Noonan, K.D. 665
Nooter, K. 666
Nowinski, R.C. 555, 667, 668, 669, 670, 671, 672, 1057
Nudel, U. 673, 674, 1002
Nussbaum, A.L. 198
Nuun, M. 30

Oakes, J.E. 452, 675, 778
O'Brien, S. 558
O'Brien, S.J. 582, 676, 677, 678, 679, 680, 681, 682, 683
O'Bryan, R.M. 344
Odaka, T. 484
Ogden, J.D. 204, 421, 684, 1069
Ogino, T. 685
Ohno, S. 686
Ohno, T. 146, 687, 688
Ohta, Y. 593, 689
Ohtsuki, Y. 238, 690
Oie, H.K. 16
Oikawa, T. 990
Okabe, H. 691, 692
Okayama, M. 485, 693
Okazaki, T. 591
Oldham, R.K. 200, 201, 609
Oldstone, M.B.A. 465, 477, 694, 695, 1084
Oleske, J.M. 528, 696
Olsen, R.G. 134, 174, 175, 176, 380, 381, 425, 426, 427, 428, 535, 536, 600, 601, 692, 697, 698, 845, 846, 847, 1088
Olshevsky, U. 699
Olson, C. 134
Olson, R. 681
Olweny, C.L.M. 700
O'Neill, F.J. 725
Opelz, G. 253
Opelz, M. 981
Oroszlan, S. 196
Orr, T. 34
Ortiz-Muniz, G. 244, 265, 571, 902, 903
Ortwerth, B.J. 141
Oshiro, L.S. 35
Oskarsson, M.K. 701, 702, 813, 1020
Ostertag, W. 249
Osugi, Y. 703
Otten, J.A. 704, 1001
Overturf, G.D. 705
Owens, R.B. 327, 359, 650, 706, 707, 708, 922, 923
Owor, R. 700
Oxman, M.N. 748

Pachmann, K. 709
Padgett, T. 89
Padhy, L.C. 195
Padmanabhan, R. 710, 710
Pagano, J.S. 345, 441, 711, 712, 876, 887, 1092
Pal, B.K. 377, 821
Panasenko, S.M. 713
Panet, A. 370, 736
Pang, R.H.L. 740, 741
Pant, K.J. 622, 623
Papadopoulos, N.M. 714
Papamatheakis, J.D. 715, 863, 864, 865, 866, 867, 868
Papas, T.S. 60, 166, 716, 717, 718, 719, 720, 721, 722, 931, 1067
Paran, M. 1075
Paranjpe, M.S. 111, 723
Park, M.S. 831
Parker, J.C. 460, 724, 1094
Parkman, P. 606
Parks, W.P. 407, 561, 725, 726, 823, 873, 874, 875, 895, 1014, 1015, 1090
Pasternak, G. 618, 727, 802
Pasternak, L. 727
Pater, M. 728
Paul, F.S. 729
Pazmino, N.H. 455, 456
Pearson, G.F. 812
Pearson, G.R. 34, 730, 756, 768, 1042
Pearson, J.W. 513, 616, 734, 856
Peebles, P.T. 731, 732
Peer, G.W. 423
Peer, M. 604
Pegelow, C. 705
Pellegrino, M. 339
Penn, G. 429
Pereira, E. 488
Périès, J. 733
Periman, P. 151
Perk, K. 616, 734, 856
Perkins, V. 660
Perlin, A. 200
Perlin, E. 735
Perline, E. 151
Perry, A. 79, 471
Perry, S. 366
Peters, G. 736
Peters, R.L. 23, 504, 737, 758, 837, 961
Peters, W.P. 146
Peterson, W.D. 120, 495, 496, 497, 971
Peto, R. 738
Petrányi, G. 739
Pezzutti, M.R. 291
Pfeffer, R.I. 585
Phillips, L.A. 740, 741
Phillips, S.M. 742, 743
Pickering, R. 670
Pickett, E.A. 263, 744

Pike, M.C. 126, 142, 319, 395, 397, 584, 585, 634, 738, 745, 746, 747, 926, 927, 928
Pitha, P.M. 748, 749
Polesky, H. 430, 433
Pollack, S.B. 645, 646
Pomenti, A.A. 230, 833
Pomeroy, B.S. 729
Pomeroy, K.A. 729
Povey, S. 169, 739
Powars, D.E. 705
Powell, K.L. 184, 750, 751, 763
Prather, S.O. 752, 753
Premkumar-Reddy, E. 754, 755
Prevost, J.-M. 756, 1042
Price, P.J. 622, 755, 757, 758, 759, 1034
Priori, E.S. 112, 238, 255, 760
Proffitt, M.R. 145, 415, 761, 762
Pry, T.W. 718, 719
Purifoy, D.J.M. 751, 763
Putnam, D.L. 764, 798

Qualtiere, L.F. 756
Quarles, J.M. 704, 765
Quist, K.D. 460

Rabin, H. 34, 652, 653, 756, 766, 767, 768, 1042
Rabotti, G.F. 180
Rabson, A.S. 580
Rai, K.K. 424
Ramierez, F. 673
Ramot, B. 73, 341
Ramseur, J.M. 294
Ramseyer, A. 980, 983
Rand, K.H. 769
Rangan, S.R.S. 770
Rankin, C.T. 934
Rapaport, E. 771, 772
Rapin, I. 1019
Rapp, F. 22, 243, 292, 324, 325, 354, 355, 356, 357, 554, 567, 595, 602, 619, 620, 621, 675, 685, 728, 753, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 789, 1004, 1054, 1055, 1056
Rascati, R.J. 1000
Rasheed, S. 319, 321, 322, 783, 784, 785, 786
Ravindranath, Y. 496
Rawls, W.E. 607, 608, 787
Ray, M. 453
Ray, U. 788, 929
Reagan, R.L. 1047
Reed, B. 769
Reed, C.L. 781, 789
Regan, J.D. 1048, 1093
Reichmann, M.E. 170
Rein, A. 254, 499, 790, 791
Reitz, M.S. 309, 310, 311, 312, 792, 1077
Reuben, R.C. 282, 593, 793
Reuzi, G.E. 720
Revillard, J.P. 543, 544
Reynolds, F.H. 794
Reynolds, F.K. 724
Reynolds, T.B. 260
Rhim, J.S. 167, 666, 764, 795, 796, 797, 798, 799, 962, 1033
Rho, H.M. 162, 330
Rich, M.A. 195, 304, 611, 800, 826
Richardson, L.S. 1075
Rie, E.J. 801
Rie, I.F. 801
Rieche, K. 802
Rifkind, E.A. 282, 593, 674, 689, 793, 1002
Rigby, P.W.J. 925
Rigden, E.E. 586
Riggs, J.L. 35, 359, 924, 944
Rinaldo, C.R. 157, 416, 803
Ringold, G.M. 804, 805
Rittenhouse, H.G. 1058
Ritzi, E. 806, 807, 808
Ritzi, E.M. 809
Robbins, K.C. 78, 131, 810
Robbins, M.L. 46
Robert-Gurcff, M. 811
Roberts, R. 1078
Robey, W.G. 141, 701, 702, 812, 813, 1020
Robin, M.S. 832
Robinson, H.L. 188
Roboz, J.P. 69, 70, 71, 72, 814, 815
Rodriguez, A.R. 261
Roizman, B. 292
Rongey, R.W. 314, 319, 320, 321, 322, 448, 804, 785
Rosén, A. 523, 816
Rosen, J.M. 817, 818
Roth, I.L. 945
Roth, S.L. 485, 693, 819
Rothenberg, E. 370, 820
Roulland-Dussoix, D. 89
Rowe, W.E. 724, 748, 749
Roy, M. 367, 368
Roy-Burman, P. 377, 657, 658, 821
Rubenstein, C. 1032
Rubin, F.H. 503
Ruch, D.G. 1033
Ruiz, P. 867, 869, 870
Rundell, A.K. 822
Rupp, R. 932, 933
Ruscetti, F.W. 633, 1076
Ruscetti, S.K. 561, 823
Russo, I.E. 824, 825, 826, 827, 828, 830
Russo, J. 611, 800, 824, 825, 826, 827, 828, 829, 830

Sabel, F.L. 49
Saby, J. 828
Saito, S. 831
Salaman, E.H. 1049

Salmon, J.E. 674
Salzberg, S. 832
Samagh, B.S. 227
Samii, H. 538, 882
Samuel, K. 166, 717
Santorineou, M. 1019
Saral, R. 47
Sarkar, N.H. 833
Sarma, P.S. 228, 729, 754, 755, 834
Sarna, G. 366
Sartorius, C.W. 835, 836
Sasazuki, T. 299
Saski, M. 1089
Sass, B. 23, 446, 737, 837
Saviolakis, G.A. 24, 969
Sawanobori, S. 640
Saxinger, W.C. 309, 312
Scanlon, M.D. 238, 690
Schafer, M.A. 719, 721, 722
Schäfer, W. 103, 211, 454, 455, 456, 838, 839, 840, 841
Schaffer, P.A. 132, 184, 267, 276, 285, 842, 843, 1005
Schaffhausen, B.S. 844
Schaller, J.B. 380, 381, 425, 426, 427, 428, 600, 697, 698, 845, 846, 847, 1088
Schechter, I. 848
Schenley, C.K. 765
Scherer, M.A. 197, 701, 702
Schidlovsky, G. 19, 20, 136, 352, 849
Schlesinger, M. 850
Schlom, J. 1, 178, 247, 248, 434, 616, 617, 851, 852, 853, 854, 855, 856, 857, 859, 860, 861
Schneider, R. 858
Schneider, S. 1051
Schnitzlein, W.M. 170
Schochetman, G. 247, 853, 854, 855, 857, 859, 860, 861
Scholz, A. 481
Schrecker, A.W. 398, 811
Schudel, A. 203, 272, 275
Schulter, B. 1001
Schultz, A. 1077
Schultz, R.M. 715, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871
Schwaab, G. 220, 543
Schwartz, B. 433
Schwartz, D.E. 872
Schwartz, J. 424
Schwartz, R.S. 901, 1016
Schwarz, H. 839, 841
Scolnick, E.M. 561, 725, 726, 731, 732, 823, 873, 874, 875, 895, 1014, 1015, 1090
Seebeck, T. 876, 887
Sega, M.W. 765
Segal, M. 431, 432
Sehgal, P. 194
Seigneurin, J.M. 214, 877
Seman, G. 112, 238, 690, 878
Sen, A. 879, 880, 881
Sesterhenn, K. 538, 882
Seth, P. 607, 608
Shank, P. 1025
Shanmugam, G. 331, 369
Shanmugaratnam, K. 220
Shanmugaratnam, K. 150, 910
Shannon, J. 681
Shantz, G. 390
Shapiro, S.Z. 883, 884, 885, 886
Shaw, J.E. 876, 887
Shaw, M.W. 549
Sheffield, J.B. 551, 888, 889, 890
Shen, F.W. 116, 891
Sherr, C.J. 81, 136, 137, 558, 879, 880, 892, 893, 1010, 1012
Shibley, G.P. 79, 471
Shih, T.Y. 894, 895, 1090
Shimizu, S. 321
Shlomai, J. 65, 296, 896, 897, 898
Shope, R.E. 170
Shore, E. 95, 96, 1095
Shore, S. 528, 696, 899, 900
Showalter, S.D. 361, 362
Shoyab, M. 52
Shreffler, D.C. 1057
Shulman, F. 1019
Shustik, C. 901
Siegert, W. 581, 625, 626
Siem, R.A. 430
Sigel, M.M. 244, 265, 571, 572, 573, 902, 903
Silberstein, H. 904, 905
Silva, D. 194
Silver, R.T. 190
Silver, S. 637
Silverman, T.N. 117
Silverstone, A.E. 51, 366, 606, 906
Simonds, J.A. 636, 907
Simons, M.J. 147, 148, 149, 150, 199, 220, 221, 659, 908, 909, 910, 911
Simonson, J.M. 680, 683
Simpson, W.F. 971, 972
Singer, S.J. 37
Singh, D. 911
Singh, I. 912
Singh, M. 913
Singh, V.N. 913
Sinkovics, J.G. 344, 502, 914
Sirsat, S. 195
Siu, C.-P. 915, 916
Skeen, P.P. 759
Skelly, J. 118, 917
Sliski, A.E. 268, 918
Smith, E. 407, 568
Smith, C. 398
Smith, E.J. 187, 919
Smith, G. 629
Smith, G.C. 920, 921
Smith, H.S. 359, 560, 707, 708, 835, 836, 922, 923, 924, 925
Smith, M.I. 43, 44, 45
Smith, P.G. 634, 747, 926, 927, 928
Smith, R.G. 309
Soares, H. 152

Soeiro, R. 788, 929, 976
Sohier, R. 221
Sompayrac, L. 281
Sonis, S.T. 930
Sonstegard, R.A. 719, 931
Sottong, P. 932, 933, 934
Souissi, T. 59, 543, 545
Soule, H.D. 613, 800, 1081
Spahn, G.J. 759
Spector, D. 89
Spence, C. 569
Spergel, G. 245
Spiegelman, S. 48, 146, 189, 687, 688, 806, 807, 808, 848, 935, 936, 937, 938, 939, 940, 941, 1052
Springer, E.L. 359, 360, 707, 708, 923, 924, 942, 943, 944, 945
St. Jeor, S.C. 22, 338, 357, 946, 947, 1056
Staal, S.P. 749
Staneloni, R.J. 286, 948
Stanley, A.G. 526
Starr, S.E. 528
Stavnezer, E. 181
Steeves, R. 101
Stehelin, D. 89, 949
Stehlin, J.S. 656
Steiner, M.R. 917, 950, 951
Steiner, S. 118, 917, 950, 951
Stelos, P. 930
Stephens, R. 952
Stephenson, J.R. 2, 3, 4, 5, 6, 7, 56, 57, 58, 153, 227, 228, 268, 270, 314, 409, 504, 657, 658, 691, 737, 742, 743, 794, 810, 919, 953, 954, 955, 956, 957, 958, 959, 960, 961, 1013, 1087
Steuer, A.P. 962
Stevens, I.A. 565
Stjernswärd, J. 1021
Stolfi, R.L. 807, 808
Strand, M. 39, 88, 457, 537, 557, 885, 886, 963, 964, 965, 966, 967, 968
Strauss, B. 898
Strickland, J.E. 24, 287, 386, 533, 969
Strnad, F.C. 44, 45, 970
Strober, S. 299, 300, 301
Stulberg, C.S. 971, 972
Stylos, W.A. 809, 868, 930
Sugawara, K. 330, 331, 464, 973, 974
Suk, W.A. 758, 759
Summers, W.C. 975
Sun, C. 577
Suskind, R.G. 180
Suzuki, R. 815
Suzuki, S. 484, 703
Sveda, M.M. 929, 976
Svedmyr, E. 478, 515
Sweet, R.W. 146, 688, 1052
Szakal, A. 568
Takagaki, Y. 703
Takasugi, J. 978, 979, 983
Takasugi, M. 21, 512, 529, 977, 978, 979, 980, 981, 982, 983
Takeichi, N. 46, 109, 110, 111, 258, 723, 984, 985, 986, 987, 988, 989, 990, 1089
Takemoto, K.K. 435
Tal, J. 69
Tamerius, J. 991
Tanaka, A. 663, 664, 992, 993, 994, 1080
Tanaka, M. 593, 689
Taraschi, M.E. 883, 889, 890
Tavitian, A. 995
Taylor, E.A. 84, 596, 597
Taylor, H. 127
Tegtmeyer, F. 822
Tennant, R.W. 704, 765, 998, 999, 1000, 1001, 1093
Terada, M. 593, 674, 689, 1002
Teramoto, Y.A. 1003
Teramoto, Y.S. 434
Terasaki, P.I. 246, 253, 468, 469, 523, 524, 564, 831
Tevethia, S.S. 1004
Thakkar, E. 86, 610
Theodore, T. 165
Thiel, H.J. 839, 841
Thiem, T. 72
Thomas, F.C. 622, 623
Thomas, I.A. 1094
Thompson, E.B. 590
Thor, D.E. 261
Thota, H. 502, 914
Till, M.M. 928
Tilton, J. 497
Timbury, M.C. 1005
Ting, P.C. 962
Todaro, G.J. 81, 136, 137, 205, 271, 568, 733, 879, 880, 881, 893, 918, 1006, 1007, 1008, 1009, 1010, 1011, 1012
Toni, R. 316, 443, 445, 446
Toth, E. 786
Townsend, I.E. 367, 368, 394
Traul, K. 952
Triantifellu, N. 498
Triche, T.J. 294
Trimmer, T.W. 443, 444, 445, 446
Tronick, S.R. 6, 7, 16, 691, 957, 1013
Troxler, D.R. 1014, 1015
Tsai, W.P. 197
Tsang, K.Y. 912
Tsichlis, P. 1016
Tucker, H.S. 1016
Tuffrey, M. 59
Tukei, P. 221
Turner, S. 925
Twardzik, D.R. 10, 17, 1017
Twiddy, R. 692

Uhlmann, C.H. 538, 882
Unger, E. 506
Utsinger, P.D. 1092

Vaidya, A.B. 552, 1018
Valerio, M.G. 34, 652
Valsamis, M.P. 1019
Van Blitterswijk, W.J. 133
Vande Woude, G.F. 701, 702, 812, 813, 907, 934, 1020
Van Hoosier, G.L. 460
Vanky, F. 515, 1021
Van Nie, R. 1022
Van Pelt, P.G.e. 1023
Varmus, H.E. 89, 90, 297, 804, 805, 949, 1024, 1025
Vasa-Thomas, K.A. 1026
Vass, W.C. 874, 1015
Vasudev, R. 754
Vella, P.P. 1050
Verbeet, M. 133
Veres-Molnar, S. 128
Verma, I.M. 439, 1027
Verma, R.S. 1028, 1029, 1030, 1031, 1032
Vernon, M.L. 799, 1033, 1034
Villarejos, V.M. 1050
Vitols, K.S. 398
Vlahakis, G. 617, 852, 854
Vogt, P.K. 37, 90, 99, 160, 251, 378, 448, 449, 635, 949, 1035, 1036, 1037, 1038, 1045, 1046
Vonka, V. 221
Voyles, E.A. 612, 1039
Vuillaume, M. 226

Wachtel, S.S. 245
Wagner, S.H. 598
Walia, A.S. 549, 1040
Wallace, H.J. 424
Wallen, W.C. 151, 152, 652, 653, 756, 767, 768, 1041, 1042
Walling, M.J. 30, 31, 32, 33
Wang, L.-H. 250, 251, 1043, 1044, 1045, 1046
Ward, J.M. 1047
Ward, P. 430, 431, 432
Ware, M.J. 599
Wars, I. 257
Waters, R. 1048
Watson, A. 672
Weber, G.H. 230
Wecker, E. 841
Wedderburn, N. 1049
Wee, G.B. 910, 911
Weens, J. 1016
Weese, J.L. 201
Wei, C.H. 436
Weibel, R.E. 129, 1050
Weinberg, D. 429
Weinberg-Zahlering, E. 65
Weiner, L.E. 470
Weislow, C.S. 379, 1051
Weiss, M. 1052
Weisser, A. 947
Weith, H.L. 872
Wellings, S.R. 139
Wells, P. 827, 829, 830
Wells, S.A. 423
Welsh, R.E. 465
West, D.L. 156, 1053
Westley, J. 127
Westman, A. 519, 520, 524
Westmoreland, D. 782, 1054, 1055, 1056
Wettstein, F.J. 1057
Wexler, I.B. 1031
Whalley, B.W. 122
Wheeler, K. 1024
Whitten, E.D. 547, 548
Wiernik, P. 424
Wife, E.L. 793
Wigzell, E. 739
Williams, C.J. 95, 1095
Williams, D. 873, 874, 875
Williams, E.H. 545
Williams, F. 923
Williams, F.E. 1058
Williams, F.R. 125
Wilson, C.B. 1059
Wilson, C.M. 623
Wilson, F.F. 930
Witz, I.P. 1060, 1086
Wold, W.S.M. 330, 347, 369, 464, 974, 1061, 1062, 1063, 1064
Wolfe, L.G. 85, 86, 203, 204, 272, 273, 274, 276, 421, 610, 684, 1065, 1069
Wolff, L.F. 698
Wong, F.K.Y. 294
Wong-Staal, F. 309, 310, 312, 792, 1066
Wood, P.A. 696, 900
Woodhour, A.F. 1050
Woods, W.A. 716, 862, 871, 1067
Woodside, N.J. 652, 767
Wright, J. 276, 1068, 1069
Wright, P.J. 588, 822, 1070, 1071
Wright, P.W. 87
Wu, A.M. 1072, 1073, 1074, 1075, 1076, 1077
Wu, M. 1078
Wustrow, F. 538, 882

Yagi, H. 643, 644
Yajima, Y. 664, 993, 1079, 1080
Yakoleva, L. 203, 272, 273, 767
Yamamoto, K.R. 805
Yang, N.S. 1081
Yang, W.K. 436, 437, 1001
Yap, J. 148
Yefenof, E. 477, 1082, 1083, 1084, 1085, 1086
Yeger, H. 1087
Yeh, J. 20, 952

Yoffey, J.M. 73
Yohn, D.S. 174, 175, 176, 380, 488, 600, 601, 847, 1088
Yong, N.K. 911
Yoshida, H. 238
Yoshida, M.C. 1089
Yoshimura, M. 485, 693
Young, D.M. 1047
Young, H.A. 895, 1090
Young, J. 434, 852
Young, L. 283
Young, L.J.T. 140, 1091
Yount, W.J. 1092
Yuasa, Y. 484
Yuhas, J.M. 1093
Yunker, C.E. 1094

Zacharchuk, C.M. 890
Zacharias, C. 589
Zachrau, R.E. 94, 95, 96, 1095
Zaharov, I. 368
Zamecnik, P.C. 570, 771, 772, 872, 1096, 1097, 1098, 1099, 1100
Zanick, D. 431, 432
Zech, L. 700
Zeelon, E.P. 1052
Zeuthen, J. 169, 519, 520, 523, 524
Zeve, V.H. 1101
Zevely, E.M. 398
Ziegler, J. 25, 1102
Ziegler, J.L. 27, 1103
Zinkernagel, E.M. 1104, 1105
Zurcher, C. 666

B.

1. a. OFFICE OF BIOHAZARD SAFETY

The Office of Biohazard Safety (OBS) provides both administrative and functional services to the Virus Cancer Program (VCP). In addition to defining the risk potential to the laboratory worker working with oncogenic viruses and chemical carcinogens, the office evaluates the contractors' performance in implementing the NCI Safety Standards. Field evaluations are performed by personnel that are active laboratory scientists having expertise in laboratory safety.

Risk is evaluated on a continual basis as new data on new and existing virus isolates become available. Horizontal transmission, host range, antigenicity, virus concentration, techniques employed, type of equipment used, and the physiological state of the host are evaluated. If required, a committee of expert scientists assesses the risk potential of new virus isolates. Based on this assessment, the Office develops practical control measures to minimize risk of biohazards and disseminates this information to the Viral Oncology Program in-house personnel, VCP contractors, and the general scientific community.

The Office develops safety standards for the proper handling of viruses and assists extramural program workers in implementing them. The Office provides educational and technical assistance on cancer research safety matters and assesses risk associated with viral oncology research. The task of prescribing protective measures to prevent laboratory acquired infection in cancer virus research is often arbitrary and difficult because the oncogenic viruses currently under investigation have not yet been implicated in human cancer. However, it is still conceivable that under the proper conditions an oncogenic agent infectious for man will be isolated. It is therefore necessary that laboratory workers and their contacts be protected from exposure to oncogenic viruses. This can be accomplished by practicing proper microbiology laboratory procedures, using laboratory safety equipment, and incorporating environmental control features in facility design. The design of equipment and facilities is aided by studying air mass movement, and dispersion and dilution of aerosols found in biomedical research laboratories. Analysis of dispersion and dilution of airborne contaminants in an enclosed work space helps determine exposure levels and assess health hazards of exposure to infectious and toxic agents. Survival and transmission of oncogenic via airborne routes as well as in urine, feces, dander, etc., are being studied.

The Office serves as a focus for the collection, maintenance, and testing of sera obtained from in-house and contract research personnel. Information relevant to the individual's serology and microbiology exposure is collected, analyzed, and integrated in a computer system. The OBS provides an annual medical examination and laboratory evaluation of all in-house research personnel. This information is subsequently entered into the computer system.

To further minimize cross-contamination, periodic surveillance of VCP contract laboratories is carried out. Special attention is directed to all VCP production facilities to minimize cross-contamination and assure homogeneity

of viral reagents. Renovation and construction plans are reviewed by OBS to assure that facilities meet OBS safety standards and that the best research facilities are obtained at the least cost. All contract proposals of the VCP are reviewed by OBS to assure that protocols and facilities minimize potential exposure hazards and maximize protection against cross-contamination.

The Biohazards Research Section (BRS) of OBS is responsible for developing technical data that permit the OBS to make recommendations regarding the risk to laboratory personnel of exposure to potential oncogens such viruses and chemical carcinogens. These studies take particular cognizance of hazards associated with fluctuations in normal physiology that are routinely encountered in the average laboratory worker. The BRS has for several years been studying the interaction of hormones and type C viruses in order to determine whether there is an enhanced risk associated with certain normal physiological conditions which modify steriod levels, such as pregnancy. The BRS was first to demonstrate that oral contraceptives, as well as diethylstilbestrol, enhance the expression of endogenous oncornavirus in target tissue and that certain agents such as DDT and ionizing radiation exacerbate this situation. Concurrent viral and bacterial infections and immunosuppression can also lead to enhanced susceptibility to induction of cancer. More recently, this section has also demonstrated that chronic modification of the endocrine system brought about by ovarectomy results, in a murine model system, in a marked increase in adrenal carcinoma with an associated increase in lymphomas. Other programs carried out by this section have resulted in demonstrating that type C viruses are expressed in reproductive tissue of such diverse species as mice, cats, nonhuman primates, and man.

A logical extension of these studies has been to investigate the pathological and physiological significance of xenotropic viruses. Studies include an examination of xenotropic and ecotropic viral interactions and the biohazard potential of the xenotropes. Murine ecotropic viruses are being investigated to determine the influcence of subvirion proteins on the immune recognition process. A very specific and sensitive binding assay has recently been developed which permits the rapid detection and quantitation of cellular recoptors for leukemogenic viruses. This allows for the detection of oncornaviral infections prior to the formation of viral antigens, development of humoral antibody, and permitting the early evaluation of a potential biohazard. Xenotropic viruses are being used to determine their interaction with chemical carcinogens in heterologous hosts in which these agents replicate but are not known to induce disease. The staff collaborates with intramural and extramural scientists of the Virus Cancer Program. Studies are in progress to elucidate the mechanisms involved in host immunocompetence during endocrine imbalance in the baboon, cat and mouse. Potential beneficial interactions of type C virus expression during gestation in these species are also under study. In collaboration with segments of the chemical carcinogenesis area, risk assessment studies and the interaction of chemical carcinogens and oncornaviruses in a rat tumor system are being investigated.

Data derived from these studies will permit a more logical evaluation of exposure risk to laboratory personnel involved in viral and chemical carcinogen studies and will also permit a determination of risk associated with research of this nature to the general population.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04804-03 ADVO

PERIOD COVERED
July 1, 1976 to June 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Type C RNA-viral Expression in Mammalian Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. S. Saviolakis	Visiting Fellow	OBS NCI
Other:	J. E. Strickland	Chemist	OBS NCI
	A. K. Fowler	Head, Biohazards Research Section	OBS NCI
	A. Hellman	Chief, Office of Biohazard Safety	OBS NCI

COOPERATING UNITS (if any)
Litton Bionetics, Inc.
Fred. Cancer Res. Ctr.
Frederick, Maryland

LAB/BRANCH: Office of the Associate Director, Viral Oncology Program, Office of Biohazard Safety

SECTION: Biohazards Research Section

INSTITUTE AND LOCATION: Division of Cancer Cause and Prevention NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.2	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The expression of endogenous type C RNA-viral genes in mammalian tissues is being investigated. Current studies include: 1) the expression of viral genes during embryonic development; 2) the mechanism of induction of viral genes by estrogen hormones in the NIH Swiss mouse uterus; 3) the association between endocrine imbalance and oncornaviral genes in the pathogenesis of adrenal carcinoma in ovariectomized mice.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To elucidate the control mechanisms for expression of endogenous type C RNA viral genes during embryonic development and under physiologic stimuli, such as steroid hormones, and to determine the function that such genes may have in the control of cellular differentiation and carcinogenesis.

Methods Employed:

Type C RNA viruses were purified from culture media of infected cells by standard procedures. High molecular weight viral RNA was prepared by sucrose sedimentation velocity gradients. DNA complementary to viral RNA (cDNA) was synthesized either by means of the endogenous reaction of RNA-directed-DNA polymerase (RDDP) of purified virions or by using purified avian myeloblastosis virus RDDP and purified viral RNA as a template. cDNAs were characterized by gel filtration chromatography, alkaline sucrose sedimentation velocity gradients and cDNA-viral RNA molecular hybridization. RNA from animal tissues was prepared by the detergent-phenol method and DNA by the urea method. cDNA-RNA hybrids were analyzed by nuclease S_1 hydrolysis and viral RNA-DNA hybrids by ribonuclease hydrolysis.

Major Findings:

Sequences complementary to Rauscher MuLV (RLV) have been detected in RNA extracted from NIH Swiss embryos obtained in early-, mid-, and late gestation, and also in early-gestation embryos from the C57L and AKR mouse strains. In early gestation, there was no difference in the number of viral sequences among these mouse strains. In early NIH Swiss embryos two classes of viral sequences were detected with a relative frequency ratio of 80/1. Whether such a variation exists in other gestation stages is being evaluated with the aid of a computer statistical program, based on hybridization kinetic theory.

Sequences complementary to a xenotropic virus isolated in our laboratory from the uterus of the NIH Swiss mouse were also detected during gestation in NIH Swiss embryos, using a cDNA prepared in an exogenous RDDP assay. This cDNA detected only a single class of viral RNA in embryos, kinetically corresponding to the high frequency class of RNAs detected by the RLV cDNA. Whether this result is due to incomplete representation of viral sequences in the xenotropic probe or to the presence of viral sequences (probably of another virus) in the NIH Swiss embryos is under investigation.

Viral RNA was detected in tissues of adult NIH Swiss mice utilizing an RLV cDNA. Lower levels of viral RNA is expressed in nonproliferating tissues (brain and muscle) than in tissues with higher mitotic activity (liver, uterus). Estrogen hormones induce the expression of viral RNA sequences in uteri of ovariectomized animals to levels comparable to those detected in the embryos. Only sequences belonging to a single class of viral RNAs could

be detected in the organs of adult mice. These findings suggest, first, that the expression of viral components correlates with the proliferative activity of individual tissues; and second, that portions of the viral genome are differentially expressed in embryonic and adult tissues. The factors responsible for such selective derepression or induction of viral genes and the functional role of their products remains to be determined. Chronic estrogen hormone deprivation, induced by oophorectomy at an early age, was found to be associated with an increased incidence of adrenal carcinoma and a lymphoproliferative disease in aged NIH Swiss mice, suggesting a role for hormonal imbalance in these disorders. Cystic endometrical hyperplasia was observed in animals bearing adrenal carcinomas. Such uteri could synthesize p30 but not RDDP in response to estrogen stimulation, again suggesting the presence of differential expression of viral genes. Viral RNA expression in adrenal carcinomas and hyperplastic spleens is currently being investigated in an initial effort to study the possible viral involvement in the pathogenesis of these malignancies.

Significance to Biomedical Research and the Program of the Institute:

Previous work from this laboratory has shown that in the uterus of ovariectomized mice, viral p30 and RDDP are modulated by estrogen hormones. Mature viral particles were most frequently observed by electron microscopy in early gestation uterine decidual tissue. In the uterus of aged ovariectomized animals p30, but not RDDP, could be induced by estrogens. The induction of RNA hybridizable to viral cDNA by estrogen further supports the conclusion that endogenous proviral genes are controlled by host physiologic stimuli. The availability of well defined molecular species (proteins, RNAs) and of reagents to detect them (cDNAs, antibodies) may establish the mouse uterus as a model for the study of the mechanism of steroid hormone action and gene expression; and for the study of *in vivo* factors affecting the processing and assembly of viral components.

The expression of viral genes during embryogenesis, and the possible modulation of discrete viral mRNAs and proteins during different stages of gestation, further suggest that physiologic factors are induced in the induction of viral genes. Whether such genes are beneficial or detrimental to the host remains to be determined.

The relationship between oophorectomy and adrenal carcinoma and a lymphoproliferative disorder is certainly due, among other things, to endocrine imbalance. The role of xenotropic virus expression in the pathogenesis of these disorders remains to be determined.

Proposed Course:

cDNAs prepared from the NIH Swiss xenotropic virus will be hybridized to early gestation embryonic RNA to low Cot values. Hybridizable and non-hybridizable cDNA will be isolated and hybridized to RNAs from mid and late gestation embryos, estrogen stimulated uteri, and other normal and malignant adult tissues. These experiments may aid in determining the

nature of the viral mRNA(s) in vivo and permit the design of further experiments to determine the control mechanisms of viral gene expression and the influence of endogenous type C viral genes on host functions.

Publications:

Strickland, J. E., Saviolakis, G. A., Fowler, A. K., Kouttab, N. M., and Hellman, A.: Impaired-estrogen mediated production of type C viral DNA polymerase in aged NIH Swiss mouse uteri. Proc. Soc. Exp. Biol. Med. 153: 63-69, 1976.

Allen, P. T., Mullins, J. A., Saviolakis, G. A., Strickland, J. E., Fowler, A. K., and Hellman, A.: Direct isolation of xenotropic retraviruses from the NIH Swiss mouse uterus. Virology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04805-07 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Risk Assessment Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Hellman	Chief, Office of Biohazard Safety	OBS NCI
OTHER:	A. K. Folwer	Head, Biohazard Research Section	OBS NCI
	J. E. Strickland	Biochemist	OBS NCI
	P. T. Allen	Microbiologist	OBS NCI
	G. A. Saviolakis	Visiting Fellow	OBS NCI

COOPERATING UNITS (if any)

Southwest Foundation for Research and Education San Antonio, Texas	Ohio State University Dept. of Veterinary Med. Columbus, Ohio	Litton Bionetics, Inc. Fred. Cancer Res. Cntr. Frederick, Maryland

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.8	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS XXX(b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are designed to contribute technical information on which to estimate risk assessment in man when exposed to oncogenic viruses, chemical carcinogens and environmental carcinogens. Model systems consisting of rodents, feline and primates are being used to evaluate risk based on modification of the normal physiological status of the host. In particular, the influence of pregnancy, hormonal imbalance and exposure to ultraviolet light and the subsequent DNA repair process are being investigated. The expression of xenotropic virus during hormonal imbalance, DNA repair and other fluctuation in host and cellular functions are being studied to further ascertain the risk of such states to the laboratory worker.

PHS-6040
(Rev. 10-76)

Project Description

Ojbectives:

1. Determine the risk to the host during U.V. induced, DNA repair, the potential for errors in DNA prone repair as well as exposure to oncogenic viruses and chemical carcinogens during physiological imbalance.

2. Evaluate the significance of xenotropic and ecotropic type C viruses in normal physiological and developmental processes.

Methods Employed:

Cell and viral exposure to calibrated energy levels of U.V. radiation and subsequent evaluation of replication repair is studied in in vitro cell cultures. Animal exposure to type C viruses during states of endocrine imbalance and subsequent response is measured by focus, radioimmuno-, and enzyme assays as well as hybridization. Similarly animal exposure to carcinogens is evaluated by tumor induction as well as cellular and humoral immune capacity.

Major Findings:

Exposure of non-producer cells to U.V. radiation brings about an efficient induction of type C xenotropic virus. DNA repair of radiated cells induces super induction of oncornavirus, subsequently permitting an evaluation of error prone DNA repair leading to mutation and possibly oncogenesis. Activation of xenotropic virus may be a protective mechanism for the host during error prone U.V. repair, as well as during other environmental induced malignancies and maybe potentially highly significant. Evidence of such a possible function for xenotropic viruses is seen in the capacity to appreciably reduce the incidence of DMBA induced mammary tumors in rats. A possible mechanism being the ability of these types of viruses to act as multifunctional antigen by providing immunological protection against oncogenic viruses as well as chemical induced transformation, perhaps by inducing immunity to cross reacting tumor antigens. It has similarly been observed, that as in man, nonhuman primates, rodents, and cats express xenotropic virus in the placenta and fetus during stages of gestation, suggesting a physiological function for these viruses.

Significance to Biomedical Research and the Program of the Institute:

The observed enhance activation of oncornavirus induced by environmental factors such as U.V. light suggest multifacet possibilities. In the bacterial phage system, phage activation and subsequent cellular error prone DNA repair leads to phage mutation. In the case of xenotropic virus mutations, this could lead to the establishment of our currently well recognized ecotropic viruses who possess the capacity for oncogenesis. The potential significance that xenotropic virus play in host physiology is further suggested by their

broad species range and our observed capacity for such agents to modify the immune process and protect against chemically induced carcinogens. The suggested relationship between cellular differentiation and viral gene expression gain further credence by the observed elevated xenotropic viral expression during pregnancy in the mouse, cat, nonhuman primate, and man. The significance of these observations can be interpreted both as bringing about possible beneficial as well as potential hazardous consequences to the host, possible dependent on cofactors such as host physiology and external environmental influences.

Proposed Course:

The potential for inducing error prone DNA repair in chemical as well as physical modified cells in vitro will be further evaluated. A greater definition of potential beneficial functions associated with type C viruses observed and isolated during gestation as well as their potential risk will be further defined. Information obtained will be incorporated into our continual risk assessment to man associated with laboratory manipulation of oncornaviruses and chemical carcinogens.

Publications:

Hellman, A. , Fowler, A. K., Strickland, J. E., Kouttab, N. M.: Type C Virus Modulation by Estrogens: Its Possible Biological Function. In Deutsch, E., Moser, K., Rainer, H., and Stacher, A., (Eds.): Molecular Base of Malignancy. Stuttgart, Georg Thieme Verlag, 1976, pp. 206-216.

Hellman, A., Fowler, A. K., Strickland, J. E., and Kouttab, N. M.: A Possible Physiological Function for Type C RNA Viruses. In Clemmesen, J., and Yohn, D. S. (Eds.): Comparative Leukemia Research, Basel, Switzerland, S. Karger AG, 1976, pp. 161-165.

Strickland, J. E., Saviolakis, G. A., Fowler, A. K., Kouttab, N. M. and Hellman, A.: Impaired estrogen-mediated production of type C viral DNA polymerase in aged NIH Swiss mouse uteri. Proc. Soc. Exp. Biol. Med. 153: 63-69, 1976.

Kouttab, N. M., Fowler, A. K. Strickland, J. E., and Hellman, A.: Suppression of in vitro lymphocyte stimulation in mice by uterine and placental extracts. J. Immunol. 117: 1644-1650, 1976.

Hellman, A., and Fowler, A. K.: Type C RNA virus expression in reproductive tissue: Possible biological significance. Microbiology (In Press).

Allen, P. T., Mullins, J. A., Saviolakis, G. A., Strickland, J. E., Fowler, A. K., and Hellman, A.: Direct isolation of xenotropic retraviruses from the NIH Swiss mouse uterus. Virology (In Press).

Weislow, O. S., Schneider, S., Heberling, R. L., Kalter, S. S., and Hellman, A.: Autogenous homoral immunity to baboon xenotropic endogenous type C virus. J. Natl. Cancer Inst. 57: 561-565, 1976.

Kalter, S. S., Heberling, R. L., Barker, S. T., Weislow, O. S., and Hellman, A.: Baboon (papio cynocephalus) oncornaviruses. The Cold Spring Harbor Symposium: RNA Tumor Virus Mtg. 1975, (In Press).

Heberling, R. L., Kalter, S. S., Eichbey, J. W., McCullough, B., Rhein, J. S., and Hellman, A.: Fibrosarcomas in dogs and non-human primates induced by a baboon type C virus--murine sarcoma pseudotype. Science (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04807-07 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunocompetence and Susceptibility of Animal Systems to Oncogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. K. Fowler	Head, Biohazards Research Section	OBS NCI
Other:	A. Hellman	Chief, Office of Biohazard Safety	OBS NCI
	D. R. Twardzik	Biochemist	OBS NCI
	J. E. Strickland	Chemist	OBS NCI
	P. T. Allen	Microbiologist	OBS NCI

COOPERATING UNITS (if any)
Ohio State University, Dept. of Veterinary Med., Columbus, Ohio
Litton Bionetics, Inc., Fred. Cancer Res. Ctr., Frederick, Maryland

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION
Biohazards Research Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.4	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The influence of host physiology on type C RNA virus expression and their participation in tumorigenesis is being examined. A rapid and sensitive assay for detecting and quantitating small differences in host cell X virus interaction, complemented by immunological and hormonal manipulation of virus expression in vivo are being used as a model system to comprehensively examine physiologic and other factors influencing viral carcinogenesis.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The objectives of this project are to develop and examine model systems for: (1) defining and characterizing physiological and immunological factors that modify the susceptibility of the host to tumorigenesis and the mechanism by which oncornavirus contribute to this process, and (2) determining the significance of type C virus information in normal tissues and the relevance of hormonal and/or immunological events modifying its expression.

Methods Employed:

Oncornavirus expression is determined by radioimmunoassay, immunofluorescence, gel electrophoresis, RNA-directed DNA polymerase activity, electron microscopy and tissue culture techniques. Cell binding and transformation assays using lymphoid cells are used to examine virus/viral protein X cell interactions. These assays are used in combination with a variety of in vivo and in vitro parameters of host immunocompetence to assess the influence of physiologic and other stimuli on oncornavirus expression and pathogenesis. These include the in vivo maternal/conceptus interactions and delayed hypersensitivity reactions, as well as in vitro one-way and two-way mixed lymphocyte reactions and the response of lymphocytes to specific and non-specific antigenic stimulation.

Major Findings:

The major envelope glycoprotein (gp69-71) purified from Rauscher leukemia virus (R-MuLV) binds efficiently to murine lymphoid cells, but not to lymphoid cells from other species. Binding of [^{125}I] gp69-71 to splenic and thymic cells is initially linear, temperature dependent and requires Ca^{++}. At 37°C, binding occurs rapidly and 50% of the maximum bound level is attained within ten minutes. In contrast, at 0°C binding proceeds much slower, with 50% saturation occurring at approximately 100 minutes. Maximum binding level at both temperatures is similar. Binding of RLV gp69-71 is competitively inhibited with unlabeled glycoprotein, but not by R-MuLV p30, partially purified murine xenotropic virus glycoprotein or several other unrelated proteins. SDS-PAGE profiles of the gp69-71 after binding to lymphoid cells is indistinguishable from pre-bound profiles. Antibody to RLV gp69-71 prevented binding; whereas normal serum and antimurine IgG and IgM sera had no effect. The assay is sensitive for detecting the receptor activity of approximately 10^4 cells. Binding is proportional up to 2.5 x 10^5 cells per ml and plateaus above 10^7 cells per ml.

The specificity and sensitivity of this assay permits the rapid detection and quantitation of small differences in the binding characteristics of different cell types for viral envelope glycoprotein; thus providing a model system to closely examine physiological, immunological and genetical factors influencing cell X virus interaction. Additionally, it serves as an assay system for the detection of cellular receptors for leukemogenic

viruses and is presently being used to isolate and characterize RLV gp69-71 membrane receptor solubilized from BALB/c thymic cells.

Preliminary studies indicate that lymphoid cells, in general, exhibit a much greater binding capacity (10 to 100X) for RLV gp69-71 than non-lymphoid cells (liver, sperm and red blood cells), and in most cases, those derived from the thymus bind significantly more radiolabeled glycoprotein than either splenic or peripheral white cells. Significant strain differences have also been observed in gp69-71 binding potential, with binding capacities ranging from approximately 0.2 to 5 x 10^4 gp69-71 molecules per thymic cell. Although other factors appear involved, reduced binding to lymphoid cells is observed in mouse strains known to synthesize endogenous Class I type virus (i.e. AKR). Presumably this occurs by the in vivo "partial" saturation of cellular receptors for ecotropic virus glycoprotein by the endogenous viruses. This is further evidenced by the marked reduction of gp69-71 binding to lymphoid cells obtained from mice exogenously infected with either Rauscher or Friend leukemia virus. Following RLV infection, reduction in the binding capacity of splenic cells is evident within 7 days (65% of controls), and thereafter rapidly decreases, binding only 20% of control levels after 14 days. Binding level of thymic cells was also reduced by RLV infection, however, significant decreases were not observed until 14 days post infection (60% of control). After four weeks binding of RLV gp69-71 to thymic and splenic cells was reduced to approximately 10% of control binding levels.

Of particular interest is the observation that the in vitro cellular mediated blastogenic response of murine lymphoid cells is modified by high-speed freeze-thaw supernatants of Rauscher leukemia virus. Further purification of these proteins revealed that envelope glycoprotein (gp69-71) stimulated transformation (2 to 3X) but had no effect on mitogen (phytohemagglutin-PHA; Concanavalin A-ConA: lipopolysaccharide-LPS) or mixed lymphocyte induced blastogenesis. Viral core protein (p30) had no effect on lymphocyte transformation. In contrast, a component of the protein fraction chromatographing in the region of myoglobin was strongly suppressive to PHA, ConA and mixed lymphocyte transformation but not to LPS stimulation. Experiments are in progress to characterize the small molecular weight component(s) responsible for the observed suppressions and determine its relevance in the immunosuppression observed during leukemogenesis.

In collaborative studies with Dr. Richard Olsen, OSU, the expression of xenotropic feline virus (RD114) p28 has been characterized in cat placental and fetal tissues. Similar to the trends noted previously by our laboratory using the mouse model, expression of the major core protein is high in the early placenta (<30d) but then dramatically decreases. p28 expression in fetal tissue is generally much lower, however, the thymus gland appears to be an exception.

Significance to Biomedical Research and the Program of the Institute:

The essential role of host immunity, as dictated by genetic and environmental factors, in the etiology of some if not all cancers, has become increasingly apparent during recent years. The concept that a decrease of natural immunity processes under certain environmental and/or physiological stresses precedes or is concomitant with infection by oncogenic viral agents is not new, although it remains poorly defined. The frequent implication of host physiology in the development and pathogenesis of cancer, as well as its involvement in host immunocompetence, points to the need of further research directed toward elucidating the biochemical mechanisms and the degree of their participation in these phenomena as a prerequisite to the development of means to control human cancers.

Proposed Course:

The development of a rapid and sensitive method for detecting and quantitating differences in the binding characteristics of host cells for viral envelope glycoprotein, complimented by immunological and hormonal manipulation of virus expression in vivo provides a model system to comprehensively examine physiological factors influencing cell X virus interactions and their relevance in tumorigenesis.

Publications:

Hellman, A., Fowler, A. K., Strickland, J. E., and Kouttab, N. M.: Type-C Virus Modulation by Estrogens: Its Possible Biological Function. In Deutsch, E., Moser, K., Rainer, H., and Stacher, A. (Eds.): Molecular Base of Malignancy. Stuttgart, Georg Thieme Verlag, 1976, pp. 206-216.

Hellman, A., Fowler, A. K., Strickland, J. E., and Kouttab, N. M.: A Possible Physiological Function for Type-C RNA Viruses. In Clemmesen, J., and Yohn, D. S. (Eds.): Comparative Leukemia Research, Basel, Switzerland, S. Karger AG, 1976, pp. 161-165.

Strickland, J. E., Saviolakis, G. A., Fowler, A. K., Kouttab, N. M., and Hellman, A.: Impaired estrogen-mediated production of type C viral DNA polymerase in aged NIH Swiss mouse uteri. Proc. Soc. Exp. Biol. Med. 153: 63-69, 1976.

Kouttab, N. M., Fowler, A. K., Strickland, J. E., and Hellman, A.: Suppression of in vitro lymphocyte stimulation in mice by uterine and placental extracts. J. Immunol. 117: 1644-1650, 1976.

Fowler, A. K., Strickland, J. E., Kouttab, N. M., and Hellman, A.: RNA tumor virus expression in mouse uterine tissue during pregnancy. Biol. Reprod. 16: 344-348, 1977.

Hellman, A., and Fowler, A. K.: Type C RNA virus expression in reproductive tissue: Possible biological significance. Microbiology (In Press).

Allen, P. T., Mullins, J. A., Saviolakis, G. A., Strickland, J. E., Fowler, A. K., and Hellman, A.: Direct isolation of xenotropic retraviruses from the NIH Swiss mouse uterus. Virology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04816-03 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Dispersion and Dilution of Air Contaminants in a Ventilated Space

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: D. L. West Environmental Health Engineer OBS NCI
OTHER: None

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.1	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Objectives of this research project are (1) to characterize the dispersion and dilution of gaseous and small-particle aerosol contaminants within a confined, ventilated space that simulates a biomedical research laboratory, or other work space, and (2) to study the dispersion of contamination in laboratories and work spaces for diverse conditions of ventilation, contaminant release, local exhaust, safety equipment, occupancy and personnel activity.

To accomplish the above task, a tracer gas is released into a test space and real-time measurements of gas concentration are made and recorded for various sample points. Data are analyzed to reveal tracer gas movement and dispersion. The results are applicable for estimating personnel exposure levels and for assessing facility and safety equipment design parameters.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Objectives of this research project are (1) to characterize the dispersion and dilution of gaseous and small-particle aerosol contaminants within a confined, ventilated space that simulates a biomedical research laboratory, or other work space, and (2) to study the dispersion of contamination in laboratories and work spaces for diverse conditions of ventilation, contaminant release, local exhaust, safety equipment, occupancy and personnel activity.

Methods Employed:

A tracer gas is released into a test space and real-time measurements of gas concentration are made and recorded for various sample points. Data are analyzed to reveal the characteristics of tracer gas movement and dispersion.

Major Findings:

After a hiatus of nine months, due to lack of technical support, research resumed in pursuit of project objectives. The effect of changing the values of specified ventilation system parameters were studied. Preliminary qualitative studies of the effect of relative air pressure between laboratories and access corridors suggest that maintenance of neutral pressure is virtually as effective in controlling the migration of contaminants from a laboratory as maintaining the laboratory at negative relative pressure.

Modifications to the tracer gas analyzing system are being made which will allow the integration of sample levels over time. This modification is essential to assess importance of very large, momentary fluctuations in concentration (i.e., spikes in concentration) that have been detected in previous and current studies.

Significance to Biomedical Research and the Program of the Institute:

The dilution of an air contaminant in an enclosed work space is an important consideration in determining exposure levels and assessing health hazards of exposure to toxic or infectious agents. The data generated by this project will help the health science researcher and the epidemiologist to more accurately evaluate personnel exposure to air-borne agents, and will assist the industrial hygienist and engineer to optimize the design of safety equipment and research facilities. The results of this work contributes to the expertise needed to improve the quality and safety of the cancer research laboratory environment.

Proposed Course:

The health significance and contamination potential of the concentration spike phenomena detected in these previous studies will be assessed. Ventilation parameters that affect the occurrence of concentration spikes will be studied with the objective of minimizing potential hazard while optimizing efficiency of engineering design. Contaminant dispersion in laboratories and work spaces will be studied for diverse conditions of ventilation, contaminant release, local exhaust, containment equipment, occupancy and personnel activity.

Publications:

West, D. L.: Contaminant dispersion and dilution in a ventilated space. ASHRAE Transactions 83(I); 1977. (In Press).

Chatigny, M. A., and West, D. L.: Ventilation rates: theoretical and practical considerations. In Idoine, L. S. (Ed).: Laboratory Ventilation for Hazard Control, Cancer Research Safety Monograph, Vol. 3. DHEW No. (NIH) 77-1293, 1977. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04911-01 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Properties of Xenotropic Viruses and Viral-Cell Interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. Strickland	Chemist	OBS NCI
Other:	A. K. Fowler	Head, Biohazards Research Section	OBS NCI
	A. Hellman	Chief, Office of Biohazard Safety	OBS NCI
	P. T. Allen	Microbiologist	OBS NCI
	G. A. Saviolakis	Visiting Fellow	OBS NCI

COOPERATING UNITS (if any)
Litton Bionetics, Inc. Oak Ridge Natl. Lab.
Fred. Cancer Res. Ctr. Oak Ridge, Tennessee
Frederick, Maryland

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION
Biohazards Research Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	1.4	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range objective of this project is to study the mechanism of expression of endogenous viral genes by normal physiological stimuli. In particular, we are concerned with xenotropic viruses and possible host cell modification of viral protein and nucleic acid composition. Present studies include: (1) Characterization of DNA found in type C virions and determination of possible functions, e.g. transfer of cellular information among cells or carrying pathogenic information. (2) Examination of the mechanism of high incidence of adrenal carcinoma and lymphoma in aged NIH Swiss mice which were ovariectomized prior to sexual maturity. (3) Determination of tRNA primer for cDNA synthesis from high molecular weight viral RNA of NIH Swiss virus M55.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To identify the role of type C viruses and viral components in normal mammalian physiology, e.g. in differentiation, reproduction, and proliferation; to examine behavior of xenotropic viruses in several hosts and to determine what modifications occur in these viruses as a result of growth in different hosts; to identify the tRNA primer for cDNA synthesis from high molecular weight viral RNA of NIH Swiss virus M55 and to determine its relationship to primers of other mammalian viruses.

Methods Employed:

Gel filtration and ion exchange chromatography, sedimentation velocity and isopycnic centrifugation have been used to purify and characterize viral proteins and nucleic acids. Labeled cDNA probes made from viral DNA and high molecular weight RNA have been used to characterize viral nucleic acids and the expression of viral genes in mammalian cells. Competitive radioimmunoassay has been used to quantitate certain viral proteins in mammalian cells and tissues, and tissue culture systems have been used to detect and identify infectious viruses and to produce some of them in quantities sufficient for biochemical analysis.

Major Findings:

A method has been developed for locating viral high molecular weight RNA in gradient fractions by utilizing its template capability for AMV DNA polymerase (reverse transcriptase). This method readily detects as little as 0.7 ng RNA, far below the level of detectibility by optical absorbance measurements. The sensitivity is considerably increased by the addition of oligo dT to the template. It is applicable also to the detection of DNA, bacteriophage RNA, and messenger RNAs.

In collaborative work with Dr. Larry Waters of the Biology Division, Oak Ridge National Laboratory, we have preliminary data suggesting that isoleucine tRNA is the primer for cDNA synthesis from the high molecular weight RNA template of M55, a xenotropic virus isolated in this laboratory from adult NIH Swiss mice. All ecotropic murine type C viruses examined to date have proline tRNA as primer, as do SiSV and FeLV and probably M7 baboon virus. In contrast RD114 virus appears to have glycine tRNA as primer.

M55 virus contains a double-stranded DNA of approximately 12S which is an excellent template-primer for AMV DNA polymerase. Heat denaturation produces single-stranded DNA of about 7S. The DNA is found in viral core preparations. In preliminary work, DNA has been found in R-MuLV, NZB virus, AMV, and RD114 virus. Levels relative to high molecular weight RNA seem to be particularly high in xenotropic viruses. Labeled cDNA probes have been made from NZB and M55 viral DNA. They do not appear to be homologous to the

corresponding high molecular weight RNA. Immunoprecipitations indicate the presence of antiviral DNA antibodies in NZB mice and in animals hyperimmunized with certain viral proteins.

In a new group of aged, ovariectomized NIH Swiss mice we have confirmed our observation of a very high frequency of adrenal carcinoma. In addition we have found a high incidence of lymphoma, a disease thought to be rare in adult NIH Swiss.

Significance to Biomedical Research and the Program of the Institute:

Viral DNA may be of interest for several reasons: (1) The DNA may be involved in autoimmune disease such as is seen in NZB mice. (2) Since this DNA is a good template-primer for reverse transcriptase, endogenous DNA synthesis reactions may produce significant amounts of probe homologous to viral DNA instead of high molecular weight RNA, leading to erroneous interpretations of hybridization results. (3) This DNA may carry cellular information between cells and even between species in the case of xenotropic viruses. (4) The DNA may be related to some viral function, e.g. transformation. (5) Viral DNA may not be functional but may provide a clue to the mechanism and site of virion assembly, e.g. if the DNA should prove to be of mitochondrial origin.

A great deal of evidence suggests a normal role for some type C viruses in mammalian physiology. The potential to carry cellular information between cells and even between species has clear implications for differentiation and evolution. The ability of xenotropic viruses to cross species barriers makes them attractive candidates as such carriers as well as potential biohazards of greater risk than ecotropic murine viruses. Possible involvement of viral DNA in murine autoimmune disease may provide a model for a similar phenomenon in human disease. Elucidation of mechanisms controlling expression of viral information may offer hope of manipulation of expression for therapeutic purposes.

Proposed Course:

Hybridization studies with cDNA probes made from virion DNA will attempt to define the source and function of viral DNA. In particular, we will attempt to determine whether viral DNA originates from nuclear or mitochondrial DNA of the host cell, whether viral DNA varies depending upon host species, and whether viral DNA can be integrated into host DNA.

Competitive radioimmunoprecipitation experiments will be done to determine the specificity of various sera for viral DNA, as well as variations in anti-DNA titer with physiological state.

Studies will continue of tRNA primers of additional viruses.

Attempts will be made to determine whether the high incidence of adrenal carcinoma and lymphoma in aged, ovariectomized NIH Swiss mice is virus related.

Publications:

Strickland, J. E., Saviolakis, G. A., Fowler, A. K., Kouttab, N. M., and Hellman, A.: Impaired estrogen-mediated production of type C viral DNA polymerase in aged NIH Swiss mouse uteri. Proc. Soc. Exp. Biol. Med. 153: 63-69, 1976.

Hellman, A., Fowler, A. K., Strickland, J. E., and Kouttab, N. M.: Type-C Virus Modulation by Estrogens: Its Possible Biological Function. In Deutsch, E., Moser, K., Rainer, H., and Stacher, A. (Eds.): Molecular Base of Malignancy. Stuttgart, Georg Thieme Verlag, 1976, pp. 206-216.

Hellman, A., Fowler, A. K., Strickland, J. E., and Kouttab, N. M.: A Possible Physiological Function for Type-C RNA Viruses. In Clemmesen, J., and Yohn, D. S. (Eds.): Comparative Leukemia Research, Basel, Switzerland, S. Karger AG, 1976, pp. 161-165.

Kouttab, N. M., Fowler, A. K., Strickland, J. E., and Hellman, A.: Suppression of in vitro lymphocyte stimulation in mice by uterine and placental extracts. J. Immunol. 117: 1644-1650, 1976.

Fowler, A. K., Strickland, J. E., Kouttab, N. M., and Hellman, A.: RNA tumor virus expression in mouse uterine tissue during pregnancy. Biol. Reprod. 16: 344-348, 1977.

Allen, P. T., Mullins, J. A., Saviolakis, G. A., Strickland, J. E., Fowler, A. K., and Hellman, A.: Direct isolation of xenotropic retraviruses from the NIH Swiss mouse uterus. Virology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04966-02 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biohazard Safety and Environmental Control Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. R. Twardzik	Biochemist	OBS NCI
OTHER:	A. Hellman	Chief, OBS	OBS NCI
	D. L. West	Environmental Health Engineer	OBS NCI

COOPERATING UNITS (if any)
ENVIRO Control Inc., Rockville, Maryland

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES XX (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) All VCP contracts scheduled for presentation and review are reviewed to determine compliance with the "NCI Safety Standards for Research Involving Oncogenic Viruses" and for identifying the need for biohazard equipment. Recommendations are made to the contractor to insure minimum hazard to the laboratory worker and maximum protection against cross-contamination. Site visits are conducted at selected VCP contract laboratories and problem areas in biohazard and contamination control are identified. Problem areas in animal holding facilities are identified and recommendations to reduce cross-contamination are made. Special attention has been given to all investigators utilizing chemical carcinogens both in in vitro and in vivo cocarcinogenesis studies. Technical biohazard and environmental control information has been provided on request to VCP contractors as well as other investigators. Technical assistance and guidance on biohazard control and containment systems and safe operational procedures has also been provided.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The Office of Biohazard Safety provides both an administrative and functional service to the Viral Cancer Program. The principal objectives are to 1) identify and assess all potential biohazards relating to laboratory facilities, equipment and personnel operational practices utilized by Viral Cancer Program research contractors and 2) to provide specific recommendations and technical assistance to improve existing safety programs, and correct identified biohazard deficiencies in order to promote maximum control of cross-contamination and a minimum hazard to the laboratory worker.

Specific objectives include:

a) evaluation of all VCP research contract proposals for physical and operational deficiencies in biohazard control and containment,

b) conduct biological safety and environmental control surveys,

c) assign probable risk associated with a specialized experimental procedure and recommend necessary biohazard implementation,

d) provide guidance and technical assistance to all program managers of the VCP including investigators in the university, industry and other government agencies,

e) review and disseminate biohazard information and educational programs

f) laison between OBS and the Office of Recombinant DNA Activities.

Methods Employed:

All contract proposals are reviewed and evaluated for the purpose of identifying biohazards associated with implementation of proposed work and for noting deficiencies in the contractors safety program. Problem areas conducive to cross-contamination are also examined. Where needed, the acquisition of biohazard equipment, modification in facility design or changes in operational procedures are recommended. A representative from the OBS attends review meetings of the individual proposals by the VCP Scientific Review Committee and presents the biohazard assessment as outlined above.

The safety and environmental control survey teams consisting of staff personnel and consultants with individual expertise in virology, biohazard safety and engineering conduct contractor site visit evaluations. The survey team is directed by a member of the OBS and is usually accompanied by the project officer who briefs the team on work scope and areas of possible concern. The team meets with the principal investigator, his staff, a facility engineer and a representative of the contractor's safety group. The principal

investigator operationally describes the project and identifies the functional laboratory units and their interactions. Information obtained includes 1) types and quantities of viruses and cell lines employed, 2) personnel and operational safety practices, 3) available biohazard equipment (Class I and II safety cabinets, autoclaves, etc.), and 4) compliance with the "National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses". The OBS representative describes the service aspect of the survey and other support activities available to VCP contractors. Subsequently, a comprehensive survey of the facility and operations is made and the team reconvenes with the principal investigator to identify major difficiencies and recommend resolution of problem areas.

Individual reports are prepared by each member of the survey team and after a general discussion a finalized survey report itemizing noted deficiencies, affirmative recommendations for their correction and a candid assessment of the contractors overall biohazard safety program is sent to the principal investigator with a copy forwarded to the project officer, contract specialist, and Associate Director for the VCP. Priority is given to all production facility contractors where protection of the product from cross-contamination is of prime importance to all recipients of VCP sponsored virus and viral reagents.

Specialized experimental procedures utilizing a moderate risk agent, pseudotypes, or a moderate or low risk agent in high concentrations are evaluated for potential risk and recommendations as to effective biohazard control are made. In vivo inoculation studies requiring both short and long term animal holding are evaluated for potential horizontal transmission, integrity of the control group, and such other factors, such as immunosuppression of the host, which may affect the validity of the experiment.

Technical assistance and guidance as related to biohazard control, safe operating procedures and facility design is also provided by the OBS to all participants of the VCP. This office responds to requests from both VCP contractors and non-contract investigators for guidance and specific recommendations for the safe implementations of new procedures and the evaluation of various contamination control systems. The assessment of risk associated while working with a particular agent under specified experimental conditions is also made.

Published technical information relating to biohazard and environmental control is provided on request to all interested investigators. Monographs prepared by support contractors are reviewed and edited for publication and are disseminated to contractors. Cassette programs addressing various aspects of biohazard control are available to contractors on a loan basis. Selected reprints and papers addressing risk assessment, unusual laboratory hazards, and biohazard control equipment are also distributed.

Major Findings:

All contract proposals, both domestic and foreign, have been reviewed and evaluated for the purpose of identifying the need for biohazard equipment and any deficiencies relating to implementation of NCI Safety Standards for working with oncogenic viruses. Requests for primary and secondary barrier systems, such as laminar flow safety cabinets and specialized biohazard equipment are considered in order to meet NCI safety requirements and the procurement of such units, if appropriate, recommended. Contractors have been identified working with moderate risk agents, high concentrations of low risk agents, or pseudotypes thereof utilizing substandard operational and facility design. Recommendations to upgrade these programs have been made. Biohazard problems unique to utilizing chemical carcinogens in co-carcinogenesis studies have been identified and interim solutions have been proposed. Hazards associated with human biopsy materials, whole blood and serum samples have also been identified and specific recommendations made.

The site visit program has pointed out that some contractors are not in compliance with the "NCI Safety Standards for Research Involving Oncogenic Viruses"; problem areas in biohazard and contamination control are still being identified and in some laboratories moderate risk agents are handled using low risk standards. In many cases contractors recognize problem areas and seek their resolution. Defective ventilation systems and pressure gradients, non-contained aerosol generation, and improper waste disposal operations are commonly noted. Poor housekeeping and storage facilities as well as animal facilities with below standard air change capacity with no provisions addressing horizontal transmission have also been noted. New problem areas are being identified in those facilities in which chemical carcinogens are being used in conjunction with animal viruses and in vivo animal cocarcinogenesis experiments. Provisions for incorporating appropriate charcoal filters into ventilation systems and biological safety cabinets, and for degradation and disposal of chemical carcinogens are being developed.

Recommendations which have minimized personnel aerosol exposure have been made to several contractors utilizing specialized experimental procedures and equipment. Biohazards associated with utilizing pseudotype viruses especially in large concentrations and in in vivo animal induction studies have been identified and recommendations and provisions to reduce potential hazards have been made. The problems of horizontal transmission of oncogenic and other microbial agents from inoculated animals to control groups or animals used in other studies have been identified and operable recommendations to rectify problem areas have been made.

The OBS has provided technical assistance and guidance on biohazard control, containment systems, and safe operating procedures to both VCP contractors and investigators in a wide variety of other research facilities. Consultations on new facility and engineering design have also been provided.

Biohazard and environmental control information and literature have been provided on request to VCP contractors and investigators representing universities, research center, industry and other government agencies.

The OBS will serve in laison with the newly established Office of Recombinant DNA Activities and will direct all VOP research contractors involving DNA recombinant studies to said office for evaluation.

Significance to Biomedical Research and the Program of the Institute:

The OBS provides the biomedical research community with a viable and functional biohazard and environmental control safety program which when properly implemented protects the researcher and the community from potential laboratory biohazards and at the same time assures the validity of the experiment.

Proposed Course:

A developmental program in the area of biohazards associated with cocarcinogenesis studies and a refocusing on initiating laboratory waste disposal practices compatible with the surrounding community will be made during the coming year.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04967-02 ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Virological Studies on Interactions of Type C Murine Viruses and Their Hosts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. T. Allen	Microbiologist	OBS NCI
Other:	A. Hellman	Chief, Office of Biohazard Safety	OBS NCI
	A. K. Fowler	Head, Biohazards Research Section	OBS NCI
	J. E. Strickland	Chemist	OBS NCI
	G. A. Saviolakis	Visiting Fellow	OBS NCI

COOPERATING UNITS (if any)	Virol. Dept., MD Anderson Hosp. Tumor Institute Houston, TX	Virus Lab. Microbiol. Dept., Georgetown Univ. Col. Med. Washington, DC	Litton Bionetics Inc., Frederick Cancer Res. Ctr. Frederick, MD
Virol. Dept. Rega Inst. Med. Res. Leuven, Belgium			

LAB/BRANCH: Office of the Associate Director, Viral Oncology Program
Office of Biohazard Safety

SECTION: Biohazards Research Section

INSTITUTE AND LOCATION: Division of Cancer Cause and Prevention
NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	1.4	2.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are to examine the interactions between type C murine retraviruses and their host cells both in vivo and in cell culture. Particular emphasis is currently being placed in (1) the possible normal functions of xenotropic viruses during pregnancy and embryonic development, and (2) the evaluation of physiological and other factors as they influence the susceptibility of the host to tumorigenesis upon exposure to tumor viruses and virus-inducing carcinogens. Virological and biochemical methodology are principally employed in this study.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To develop and study model systems for (1) evaluating the risk associated with selected physiological factors as they relate to viral carcinogenesis or cocarcinogenesis, and (2) examining the role of certain retraviruses in normal mammalian physiological functions, and (3) examining the interaction of antiviral agents with retravirus systems.

Methods Employed:

Quantitative methods for determining xenotropic retravirus concentration include: (i) the use of sarcoma-positive leukemia-negative (S+L-) culture systems to evaluate samples for infectivity by focus-induction assay. (ii) Assays for reverse transcriptase levels, (iii) radioimmunoassay for viral protein components. Extensive use has been made of linear gradient polyacrylamide gel electrophoresis (PAGE) to examine the polypeptides of selected retraviruses. Standard approved methods for animal care have been used in studies on the effect of xenotropic viruses on carcinogenesis by chemical agents.

Major Findings:

One of several xenotropic retraviruses isolated in this laboratory from the adult NIH Swiss mouse uterus was selected for further characterization. This virus, designated M55, has a higher infectivity:total particle ratio than comparable viruses heretherefore isolated, though it is similar by serological criteria to other murine xenotropic and ecotropic viruses. The major 30,000 dalton core protein (p30) and the high molecular weight glycoprotein (HMWgp) of M55 virus are distinguishable from those of Rauscher murine leukemia virus (MuLV-R) by a faster migration in PAGE. A further distinction between M55 virus and MuLV-R is the relative content of two other proteins, p12 and p15. M55 virus is relatively deficient in p15 while MuLV-R is relatively deficient in p12. Several other xenotropic viruses have also been compared with M55 virus by PAGE, including BALB: virus 2, AT124 virus, and an isolate from the NZB mouse. These viruses are similar to M55 virus with respect to relative content of p12 and p15, but are similar to MuLV-R with respect to the large glycoprotein component. Preliminary data indicate that certain minor protein components may be distinctive for particular xenotropic virus strains.

As a model system in the study of xenotropic virus effects on potential host physiology, the influence of M55 virus on chemically-induced carcinogenesis in the rat mammary gland is under study. This project is a collaborative effort involving Dr. O. S. Weislow and Dr. R. E. Shepherd of Litton Bionetics. A single inoculation of female Sprague Dawley rats with live M55 virus leads to reduced incidence of mammary carcinoma following administration of the carcinogen dimethylbenzanthracine (DMBA). Current data indicate the absence of infectious virus particles in the spleens of

virus-inoculated rats, though all inoculated animals produce circulating precipitating antibody to the viral p30 component. Antibody to p30 is also present in the tumors of those rats developing carcinoma despite virus-inoculation. Serum antibody reactive with NZB viral glycoprotein was absent in all tumor-bearing rats tested, but was present in all other virus-infected animals. Highest titers occurred in virus-inoculated tumor-free DMBA-treated individuals. This antibody did not react with glycoproteins of MuLV-R. These results suggest that cellular production of M55 viral components may modulate cellular events leading to carcinogenesis, perhaps through an immunological mechanism. Other data from experiments carried out in vitro indicate that M55 virus may replicate more readily in transformed rat cells than in cells of normal origin. If a similar preference occurs in vivo, partial expression of the viral genome may take place in preneoplastic tissues, leading to elimination of such cells by an undisclosed mechanism.

Also in collaboration with Dr. R. E. Shepherd a study of the DNA polymerases associated with rat mammary carcinomas is underway. Current data demonstrate that tumor tissue contains a DNA polymerase (DNA pol R) of 50,000 to 60,000 daltons which utilizes poly(A)·oligo(dT) as a template in preference to poly(dA)·oligo(dT). DNA pol R is also present in normal lactating rat mammary gland tissue, but at lower concentration than in tumor tissue. DNA pol R of neither tumor nor lactating mammary gland is inhibited by antiserum prepared against the MuLV-R-associated DNA polymerase. Further study is required to determine the origin and nature of this enzyme.

Significance to Biomedical Research and the Program of the Institute:

Current evidence indicates that xenotropic type C retraviruses represent a class of viruses which has persisted in certain orders of the animal kingdom for many millions of years. It is a reasonable hypothesis then that these agents or their genetic components may provide an evolutionary advantage to animals possessing them. Our data suggests a possible preventive function in environmentally induced carcinogenesis. Further investigation of this experimental model may lead to information about natural defense mechanisms in carcinogenesis. A detailed characterization of the model virus employed in this system will be valuable in the critical evaluation of any viruses or virus-related proteins and other components recovered during the study. A thorough examination of the replicative enzyme profile of the model tumor system is also essential to evaluate the role of endogenous or exogenous viruses in the experimental model, and may also generate information useful in the control of mammary carcinoma independent of the tumor/virus model.

Proposed Course:

Further investigations will be directed toward a more critical examination of the rat model for evidence of virus replication or production of viral components in a variety of tissues, and will broaden the model to determine the efficacy of other xenotropic viruses in the prevention of mammary

carcinoma. The model system will be studied in depth to examine such factors as virus dose-response, the temporal relationship between virus-inoculation and DMBA-treatment and treatment with antiviral agents. In addition the biophysical, biochemical and serological features of selected xenotropic viruses will be comparatively examined, including a determination of the effect of the producer-cell species on minor components of the virion. The study of DNA polymerases of transformed and virus-producer cells will be continued as an adjunct to studies described above, with emphasis on characterizing enzyme activities under varied reaction conditions as a mode of enzyme identification.

Publications:

Allen, P. T., Schellekens, H., Van Griensven, L. J. L. D., and Billiau, A.: Differential sensitivity of Rauscher murine leukemia virus (MuLV-R) to interferons in two interferon-responsive cell lines. J. Gen. Virol. 31: 429-435, 1976.

Allen, P. T., and Stewart II, W. E.: Evidence that detergent-reactivated interferons are not renatured. J. Gen. Virol. 32: 133-137, 1976.

Billiau, A., Heremans, H., Allen, P. T., and De Somer, P.: Influence of interferon on the synthesis of virus particles in oncornavirus carrier cell lines. IV. Relevance to the potential application of interferon in natural infectious diseases. J. Infect. Dis. 133: A51-A55, 1976.

Allen, P. T., Mullins, J. A., Saviolakis, G. A., Strickland, J. E., Fowler, A. K., and Hellman, A.: Direct isolation of xenotropic retraviruses from the NIH Swiss mouse uterus. Virology (In Press).

b. OFFICE OF PROGRAM RESOURCES AND LOGISTICS

The Office of Program Resources and Logistics, Viral Oncology Program, is responsible for planning, initiating, and maintaining a coordinated program to anticipate and meet the needs of the intramural and the extramural collaborative Viral Oncology Program for research resources and logistical support. This coordinated program includes the initiation, development, maintenance, and management of resources contracts, responsibility for the day-to-day general management and direction of all resources distribution, and the development and maintenance of a highly sophisticated computerized inventory and management information system for the research resources acquired and utilized by the overall Viral Oncology Program. The Office was established by NCI in 1972 to centralize the scientific administration and management of research resources and logistical functions and to unify these activities for the Viral Oncology Program.

Laboratory cancer research investigations carried out in the Viral Oncology Program depend on the availability of viruses, viral reagents, antisera, animals, and clinical and laboratory materials of quantity, optimal purity, viability, and potency. Research studies in an integrated program of international scope, as encompassed in the NCI Virus Cancer Program, make more meaningful and rapid progress when adequate quantities of such standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these research materials and other supportive activities through contract operations representing six general areas of activities. These include:

Activities directed toward production and characterization of purified viruses, viral reagents, and appropriate antisera.

Activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimen material.

Activities concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type animal holding facilities.

Activities directed toward the provision of specialized testing services for the examination of experimental materials.

Activities concerned with providing support for the biohazard safety program.

Activities directed toward providing laboratory facilities and services for direct support of NCI intramural research programs.

The overall requirement for research resources and the number and extent of requests received frequently exceed the availability of Program resources. This is due, to some extent, to the high cost of producing or preparing certain scarce reagents, to fiscal considerations which limit the ability to prepare every potentially necessary item, and to a valid reluctance to prepare highly specialized and expensive materials which can be utilized by only a few laboratories. Because requests do frequently exceed availability, the distribution of resources is influenced by the relative value of specific research in relation to overall Program goals. These relative values, or priorities, are determined by the VCP Coordinating Committee, the VCP chartered Scientific Review Committees (peer review), the OPR&L Advisory Group, and the VOP Laboratory, Branch, and Office Chiefs. The distribution of resources is patterned on the evaluations and recommendations of these groups.

The principal advisory committee assisting OPR&L in carrying out its varied functions is the Program Resources and Logistics Advisory Group. The OPR&L Advisory Group constitutes a standing intramural committee to provide support and make recommendations concerning resources and logistics matters, and to conduct appropriate reviews for those contracts administrated by OPR&L. The Advisory Group is chaired by the Chief, OPR&L, and is responsible to the Office. The membership includes scientist-managers representing the Collaborative Research Branch, VOP, (VCP), a representative of the VOP Office of Biohazard Safety, and also representatives from the four major intramural Laboratories of Viral Oncology Program. The current membership of the Group is listed in a previous section of this report concerning Program committee membership.

During the past fifteen months, OPR&L has coordinated the distribution of a wide variety of biological, chemical, and materiel resources to NCI intramural investigators and VCP participants. The amount of material processed has been extensive. To illustrate the scope of activity, during this period the purified and concentrated products from approximately 60,000 liters of tissue culture-grown viruses, propagated in over 40 different cell lines, were distributed in over 1250 shipments to over 250 participating laboratories throughout the world. Additionally, over 1,000 grams of a single virus and its molecular components, not propagated in tissue culture, were sent to over 500 different investigators in over 250 shipments. As directed by OPR&L, the Cell Culture Laboratory distributed over 1250 cell culture seed stocks to over 200 individual scientists in over 200 separate shipments, and analyzed over 175 individual cell cultures submitted by approximately 50 laboratories for cell-line purity and species identification. Furthermore, the Program repositories handled or shipped out over 37,000 individual items. In addition to these activities, the Office has coordinated the distribution of a variety of resources to Russian, French, and Japanese scientists in keeping with formal U.S. or NCI international agreements with those countries covering the mutual exchange of cancer research materials. The materiel supplied included purified and concentrated viruses of murine, feline, avian, and primate origin; specialized viral proteins and antigens; normal and infected tissue culture cell lines;

various tumor-derived cells; a wide range of standard reagents and chemicals for tissue culture utilization; a wide range of antisera prepared in a variety of host animals; substances for chromatographic separations; adjuvants; and various other materials and substances.

An additional responsibility of OPR&L is the monitoring and evaluation of the virus and reagent production and purification activities of Viral Oncology at the NCI-Frederick Cancer Research Center. OPR&L is also responsible for the general management of the distribution of viruses, viral reagents and components, and cell cultures prepared at FCRC and distributed to VOP collaborating laboratories. During this report period over 20,000 liters of virus materials were produced and purified, and over 16,000 ml. of various virus concentrates were released to investigators as directed by this Office. All Program requests for viruses available from FCRC have been met and approximately 2,000 ml. of virus concentrates remain available at the FCRC virus repository to meet any additional needs.

The OPR&L is also concerned with the preparation of an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. These include viruses, antisera, human specimens, animal resources, special materials, and various services. Usually the information provided for each item includes designation, origin, and processing procedure. This year, as in prior years, a revised new edition of the catalog was prepared and distributed to Program participants. Loose-leaf binding, adapted earlier, was retained to permit efficient future updating of information, and the format and contents were again revised to expedite the recovery of items or services and reflect current research interests. As noted in the catalog, an element of the OPR&L is concerned with the maintenance of a computerized central inventory for the sera, tumor tissue, cell out-growths, and other human specimen materials continuously being acquired by the Program. The central inventory facilitates matching investigators' requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

The Resources Data Management unit within the OPR&L continued to assist in the automated retrieval and inventories of Viral Oncology Program resources, computer-systems planning consultation support, and automated resources analysis and management support. The automated inventories of the VOP resources include the OPR&L serum collection, the OPR&L human tissue collection, and the collections of the satellite resources systems. The OPR&L RDM unit maintains a computerized inventory of approximately 75,000 human sera specimens and approximately 3,000 freshly frozen malignant and normal human tissue specimens maintained in local and regional repositories. The RDM unit manages the continuous input, updating, and distribution data for these specimens stored for utilization by scientific investigators. Clinical and demographic data relevant to specimens distributed are provided to recipient investigators to assist in the analysis of test results. Furthermore, support is provided for previously developed and installed inventory systems in laboratories participating as regional resource repositories for Viral Oncology. These include the Cell Culture Laboratory of the University of California at the Naval Biosciences Laboratory, and

also the antisera production facility at the Huntingdon Research Center. Additionally, automated computer support is provided for the specimen inventory of the Laboratory of Medical Oncology, NCI. Inventory listings are provided for each of the satellite repositories maintained in the OPR&L automated inventory system, and additional ad hoc reports are prepared as required.

The OPR&L RDM unit has developed compatible automated systems and codes for human specimen material in all institutions participating in the VOP either as collection or acquisition laboratories, or as repository or distribution centers. This system includes the capture and automation of complete demographic, clinical and laboratory information on specimens and specimen donors. The goal is a central specimen inventory at OPR&L, making available in a uniform manner for all of Viral Oncology, the many specimens held at various repositories.

Additionally, the RDM unit maintains an automated management system for data relevant to the distribution of research resources managed and administered by the OPR&L. Also maintained is a data system for tracking the distribution of all virus products regardless of production source or recipient identity and location. Currently, the RDM unit is involved in planning and implementing comparable systems for the management of human specimen material. Following this, the distribution of other resource materials will be incorporated into the automatic management system.

Within the Office of Program Resources and Logistics, principal support is provided by three staff scientists, a systems analyst/computer programmer, and two secretaries, who all assist in responsibilities for the management of the collaborative contract program concerning research resources, and for the coordination of resources distribution.

c. FREDERICK CANCER RESEARCH CENTER

Introduction: The Viral Oncology (VO) Program at the Frederick Cancer Research Center (FCRC) will have essentially concluded five and one-quarter years of operation on September 25, 1977. The overall objective of the VO/FCRC Program is to conduct a comprehensive investigation on oncogenic and suspected oncogenic viruses and their interactions with host cells to determine the potential carcinogenic or co-carcinogenic role these agents may play in the neoplastic process, and to develop therapeutic and preventive measures for their control.

Following an interval of program assessment and the subsequent implementation of several preliminary steps, one year ago last June NCI/VO introduced a plan that provided for an extensive reorganization of the VO effort at its Frederick facilities. In essence, this plan called for doing away with the Task or Project structure in favor of one that provided for a more flexible and comprehensive response to NCI/VO Program needs and requirements. Thus, the program essentially was to consist of a substantial new research effort along with an increased resource activity to accommodate the increased emphasis on the former. The new structure consisted of RNA Virus, DNA Virus, and Disease Control Laboratories and a Viral Resources Laboratory. Of critical importance from the standpoint of the reorganized program development, was the addition to the Contractor's staff of the Director of Viral Oncology (DVO). This individual, well recognized in the scientific community for his scientific and administrative capabilities, joined the VO/FCRC program in January of 1976. The arrival of the DVO marked the positioning of an important cornerstone around which NCI's plan for significant development of the VO/FCRC program could be brought to fruition, and since that time many important achievements have been made.

Program-wise, a fifth Laboratory effort was initiated: The Director of the VO Program, which involves work in immunochemistry and in oncornavirus immunobiology, as important adjuncts to research in the other Laboratories. Additionally, the DVO has successfully attracted capable scientific personnel for strengthening new and ongoing effort. Of great importance also was the initiation of a new era of cooperation between Contractor and on-site NCI senior Staff investigators, resulting in each performing a mutually beneficial role within the VO/FCRC sphere of activities. Moreover, valuable collaborative efforts have been initiated with a number of recognized outside scientists whose expertise will advantageously augment the VO effort. Also, with members of the NCI and Contractor's staff, facilities were planned and renovated and/or improved to provide highly suitable laboratories for conducting the VO program.

With completion of the laboratory renovations near, as well as recruitment of the staff, who are now targeted in a meaningful manner toward accomplishing the objectives at-hand, it is evident that plans envisioned for the restructured VO/FCRC Program are well on their way toward effective implementation.

Source of Program Direction: Program direction is derived from the Associate Director for the Viral Oncology Program with recommendations from a number of outside and in-house advisory groups. Outside groups that provide formal advice and/or technical reviews include the Virus Cancer Program Advisory Committee. This group, convening several times throughout the year, provides recommendations to the Virus Cancer Program; this impacts upon VO operations at Frederick because of the intimate association between the VO/FCRC and the VCP. A second group, Virus Cancer Program Scientific Review Committee B, provides for a peer review group consisting of a minimum of six recognized outside experts, in viral oncology, who meet annually for the specific purpose of evaluating technical aspects of the VO/FCRC Program. In-house recommendations are obtained on an as needed basis from the Scientific Coordinator for Viral Oncology, in residence at Frederick, a number of NCI VO Program monitors, and other senior NCI staff personnel who provide specialized individual or ad hoc committee assistance.

Mechanism for Program Review and Evaluation: All work conducted within the VO Program at FCRC is reviewed on a continuing basis for the purpose of attaining and maintaining excellence of programs that have been established, and to insure rapid response of VO/FCRC operations to needs related to Viral Oncology and NCI program objectives. It is the responsibility of the SCVO to oversee implementation of NCI Viral Oncology programming at FCRC and to facilitate and participate in a number of review and evaluative processes that are necessary to insure maximal qualitative and quantitative accomplishment.

The first line of review is the periodic evaluations by NCI Monitors of VO/FCRC work performed by the Contractor. The Monitors, one functioning for each of the Laboratories, were carefully selected on the basis of their scientific background and experience related to the work conducted. It is the responsibility of the NCI Monitors to discuss all aspects of the work on a monthly basis with the appropriate Contractor manager and to submit bi- or tri-monthly written evaluations on progress made and/or problems that have arisen during that period of time. The written evaluations are reviewed by the SCVO who provides these together with his own comments for transmittal to appropriate NCI authorities and to the Contractor for their consideration. Reviews conducted in this manner provide a rapid system of communication whereby the Contractor can be commended for progress in a given area and/or apprised of shortcomings in the work performed so that, in the event of the latter, these can be corrected without undue delays. In addition, the monthly evaluations are cumulatively judged semiannually, forming the basis upon which the Contractor's award fee is determined. Based upon input from the SCVO, final fee recommendation is made by the Award Fee Board, consisting of senior NCI administrative staff members, for final approval by the Director of NCI. This year for the first time, a portion of the VO work functioned on a fixed fee basis, namely the new effort in Immunochemistry and Immunobiology mentioned above; it was therefore not subject to evaluation by the Award Fee Board, as were the other portions of the program. In addition, the VO/FCRC outside peer review group conducts annual technical reviews of the Contractor's effort, provides evaluative input regarding accomplishment of objectives, and offers recommendations as to programming.

General Program Consideration and Objectives: The overall objectives of the VO/FCRC Program are to conduct investigations on oncogenic and suspected oncogenic viruses and their interactions with host cells to: (i) determine whether viruses comparable to those known to induce cancers of laboratory and domestic animals are associated with, indicative of, or instrumental either alone or as co-carcinogens in inducing the development of human cancers, (ii) define the character and mode of action of virus and/or viral components in their relationships to tumor formation, and (iii) to devise therapeutic and preventive measures for their control. As described in last year's annual report and above, a restructuring of the VO/FCRC Program had provided for a significant departure from the original "Project" type of semi-delineated effort, resulting in plans for all work to be conducted within the framework of the five major Laboratory programs. This has promoted increased opportunity for the VO/FCRC effort to be performed as a total integrated activity allowing for a high degree of flexibility in response to overall NCI Program needs, and broad latitude in creating and pursuing fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts.

In considering VO/FCRC program objectives, descriptions of research and resource activities, and funding requirements, it is of importance to note that VO/FCRC operations continue to consist basically of two types of effort. The first type is characterized as being completely Contractor oriented; this means that the work is executed only by Contractor personnel utilizing Government owned equipment in NCI facilities. Scientific investigations are performed and resources provided as required by NCI, for a stipulated period of time. The second type of effort is also related to NCI research requirements but it is performed with professional and technical NCI personnel working collaboratively with professional and technical Contractor personnel on investigations that are pertinent to Viral Oncology programs conducted both at Bethesda and FCRC. Both types of work were performed within either the RNA Virus Laboratory, DNA Virus Laboratory, or the Viral Resources Laboratory; last year, for the first time, all work in the Disease Control Laboratory was conducted on a collaborative basis.

More specifically, lines along which the VO/FCRC program proceeded during the past year are as follows:

RNA Virus Laboratory. To study control of expression and natural history of type B viruses (mouse mammary tumor virus, Mason-Pfizer, other non-c retraviruses), relation of virus expression to disease, attempt to control disease in natural host; to relate information and utilize reagents obtained to understanding human breast cancer.

To study biochemical and cellular factors concerned with the regulation of and assays for type C virus expression; to develop special mutant cell lines and somatic hybrids to study control of virus synthesis; to isolate functional viral proteins.

To conduct molecular biology investigations with types B and C oncornaviruses, generating specific probes for molecular hybridization for studies on viral interrelationships, viral replication, and transcription of the viral genome in normal and transformed cells.

To provide necessary immunology and molecular biology support for an ongoing collaborative program with NCI personnel investigating interrelationships among type C virus activation and normal host physiological processes, as possible mechanisms by which viruses influence or can be influenced by host-tumor interations.

DNA Virus Laboratory. To conduct in vivo and in vitro studies with primate herpesviruses, investigating the disease process involved in tumor development at the cellular and molecular level, to analyze the behavior of tumor viruses, tumor and transformed cells, immunological activity, and other host factors for the role they play in tumorigenesis, including the application of such studies to man.

To study the isolation of single stranded HSV DNA probes for determining whether specific regions of the HSV genome are present in HSV transformed cells; to determine the polypeptide pattern of HSV-1 and -2 nucleocapsids to identify and characterize individual specific viral antigens, for use in studies on transformed and tumor cells of experimental and natural origin.

To provide necessary support for collaborative studies with NCI investigators on the mechanism of cell transformation by herpesviruses and their possible involvement as co-carcinogens with other viruses or chemicals, and for studies on alterations that occur in transformed cells, focusing on cellular enzymes, processes that repress or derepress normal cell components, and changes in properties of cell membranes induced by virus infection.

Disease Control Laboratory. To provide necessary professional and technical support for a collaborative program with NCI personnel to develop appropriate genetically defined animal model systems for studying the influence of endogenous virus expression on normal cell differentiation and on abnormal changes leading to malignant transformation, to quantitate and analyze translational products of type C isolates, and genetically map selected type C RNA viral genomes.

To provide necessary professional and technical support for a collaborative program with NCI personnel in evaluating various vaccines and vaccination protocols for their efficacy in preventing the expression of spontaneous, and chemically- or X-ray-induced neoplasia in animal test systems showing low to high expression of endogenous viruses.

In the Director of Viral Oncology Area, to isolate, purify and characterize by immunochemical techniques specific viral and viral induced proteins of biological, immunological, serological and virological importance to the Viral Oncology Program; to produce specific antisera to these proteins.

To conduct immunobiological surveys with reagents obtained above in model animal species and, where applicable, in man for evidence of oncornavirus expression, using radioimmunoassay and other techniques; to isolate and utilize tumor specific or related products for immunoprevention experiments in model system.

To perform necessary collaborative electron microscopic support with an NCI investigator to develop new applications for the electron beam in understanding the structure and function of virus-cell interactions.

In the Viral Resources laboratory, to conduct a major resource effort and provide facilities for the large-scale production of viruses or viral components that are necessary for performing laboratory research in the VO/FCRC or associated outside VO programs; to engage developmental research on oncogenic or suspected oncogenic viruses necessary to the VO program but for which no previously established protocols exist or for which protocol improvement is required; to develop suitable quality control support activities involving electron microscopy, tests for contamination, and other appropriate biochemical, serological and biological tests to insure maximal quality of resources.

Other ancillary objectives are as follows:

Environmental Control. To conduct a comprehensive safety and environmental control program for the Frederick Cancer Research Center and to perform applied and basic studies and literature surveys for risk assessment in support of the various FCRC operations.

Animal Breeding. To operate an animal farm for the breeding of laboratory animals to meet the needs of research programs at FCRC, and for shipment to other NCI operations as production permits.

Animal Health Diagnostic Section. To provide for monitoring the health and genetic quality of research animals at the FCRC and NCI in order to ensure the validity of animal-related research and to preclude the entry of undesirable pathogenic microbes, latent murine viruses and parasites.

Program Projections. Just prior to and throughout the past year, with the arrival of the Contractor's Director of Viral Oncology (DVO) along with the phasing in of a highly capable staff, and the completion of facilities renovations, the restructured program began to be effectively implemented into operational laboratory activities. This has resulted in subsequent scientific accomplishments that are now furnishing increased amounts of pertinent new basic and applied information on problems concerning the elucidation of the relationships of viruses to neoplastic disease relative to cause, detection, prevention and control. The fact that the Contractor's DVO has now fully assumed his responsibilities as the LBI FCRC leader of the VO/FCRC Program and that the restructured effort and modified objectives are now being implemented augurs well for a substantial period of overall relative program stability. Thus the approximate 30 percent increase in program activities predicted last year and implemented this year is not expected to change

appreciably. Any program alterations will essentially be accomplished within current funding and staffing concepts. Moreover, collaborative support of on-site NCI investigators appears to be in a similar state of stability. The establishment of programs for Drs. Hampar, Hatanaka and Stephenson are now fully in effect as is the effort for Dr. Huebner in Bldg. 522 and for Dr. Hellman in Bldg. 538.

There still remains, however, the problem of holding animals displaced by the renovations of VO's laboratories in Bldg. 560. Initially, provisions were to have been made for the housing of animals in Bldg. 567 upon being vacated by NINCDS. This provision was, in fact, a major consideration upon which the animal rooms in Bldg. 560 were converted into laboratory space. While the assignment of the first floor of Bldg. 567 for animal holding is an NCI decision, hopefully this is reaffirmed now that recombinant DNA activities will ultimately be performed in the Bldg. 400 area instead of in Bldg. 567, and it is expected that the Contractor will present a plan(s) to solve the animal holding problem once and for all without further ado.

Program Funding: During the interval covered in this report, Viral Oncology funds fully supported work conducted in the RNA Virus, DNA Virus, Disease Control and Viral Resources Laboratories, and the Director of Viral Oncology Research Area; TQ funding for this from July through September 1976 was approximately $1.55 million and from October 1976 through September 1977, approximately $7.68 million. Similarly, for Projects 4, 12 and 21, partially funded by VO, the TQ cost was approximately $139,000, and from October 1976 through September 1977 approximately $613,000. Approximately $176,000 of additional funds were allocated to the U.S. Army as Viral Oncology's TQ portion of the Interdepartmental Support Agreement and $967.924 for the period October 1976 through September 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04801-05-ADVO

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Electron Microscope Development; Studies of Viruses in Human and Animal Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: V. H. Zeve Research Biologist ADVO NCI

COOPERATING UNITS (if any) M. Gonda, FCRC, LBI; M. Hatanaka, NCI; K. Nagashima, FCRC, LBI; J. Elser, FCRC, LBI; M. Gregg, FCRC, LBI; J. Walker, FCRC, LBI; K. Hsu, Columbia Univ., N.Y.; S. Oroslan, FCRC, LBI; H. Eharman, FCRC, LBI; D. Fine, FCRC, LBI; L. Arthur, FCRC, LBI

LAB/BRANCH
Office of Associate Director, Viral Oncology Program

SECTION

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Major emphasis is placed on the development and application of the scanning electron microscope and an associated energy dispersive X-ray (EDX) system to viral oncology studies. Immunological tagging with heavy metals and immunolatex spheres reacted to specific areas of the cell surface have been studied to provide a system for the localization of viral antigenic sites as detected by SEM-(EDX). The SEM and transmission electronmicroscope (TEM) were utilized to study the ultrastructural characteristics of human normal and tumor cells and two retraviruses: equine infectious anemia virus (EIAV) and bovine leukemia virus (BLV). The TEM-equipped with a video image intensification system has been utilized to study thin sections of virus pellets in order to obtain video tape stored information for pattern recognition studies.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Under the general scope of investigating new methods to utilize both the transmission (TEM) and scanning (SEM) electron microscopes as a quantitative analytical research tool toward further understanding the structure and function of virus and virus-cell interactions.

(a) Collaborative support and quality control service for the Viral Oncology Program at FCRC and other cooperating NCI investigators.

(b) Ultrastructural studies of the surface features of human normal and tumor cells in culture by scanning and transmission EM.

(c) Immunoelectron microscopic study of the localization of viral antigens leading to the further development of heavy metal tagging for visualization and mapping by energy dispersive X-ray SEM.

(d) Ultrastructural studies of new retraviruses and their intracellular development.

Methods Employed:

Methods employed to carry out the outlined objectives includes the use of the SEM, TEM and their associated electronic devices. The SEM was interfaced to an automated particle analysis system as well as an energy dispersive X-ray detector.

The TEM having been equipped with a high resolution video imaging system was utilized to obtain video tape stored data for image analysis and enhancement of known virus particles.

Major Findings:

Currently the scanning (SEM) and transmission (TEM) electron microscopic studies have compared the ultrastructural characteristics of human normal and tumor cells in tissue culture. High resolution SEM has made it possible to visualize the three-dimensional image of the cell surface, an image unobtainable by other means. Human tumors from a variety of histopathologic types of solid tissue were established in culture. Tumor cells, as studied by SEM, continue to show increased surface activity in the form of many types of specific structures as compared to the smooth surface of normal cells. Tumor cells were compared to normal human fibroblasts and normal human epithelial cells grown in vitro at low density, normal human fibroblasts exhibited some surface features characteristic of human tumor cells. At higher cell density there was no extensive surface modification. The surface topography of normal human epithelial cells was similar to that of the normal human epithelial cells was similar to that of the normal fibroblasts. The availability of human tumor cells possessing specific cell

surface components should make it possible to investigate the function of these surface features in more detail. In the process, the role of the differences in surface structures of normal and tumor cells in dictating malignant potential of human tumors may be more clearly defined.

Immunological tagging of virus-associated antigens on surfaces of infected cells is already possible with well established methods for light and transmission EM. Preliminary studies were undertaken to apply tags to cells which could be detected in the SEM equipped with an energy dispersive X-ray system. Recent studies were designed to utilize new immunolatex spheres for the study of the localization of two major proteins of RLV, the 70,000 MW glycoprotein (gp70) and the 30,000 MW core protein (p30), using antisera prepared in goats. Results were compared with those of indirect labeling of the same viral proteins using immunoenzyme and immunoferritin techniques in both the TEM and SEM. By all three immunoelectron microscopic procedures in the TEM, anti-gp70 was found to label the entire viral envelope and discrete areas of the cell membrane. In contrast, anti-p30 did not label the viral envelope or cell membrane of infected cells as shown by both SEM and TEM. These immunotechniques and reagents are now being used to screen other murine and non-murine type C virus for immunological relatedness.

Two viruses with retravirus properties were studied: equine infectious anemia virus (EIAV) and bovine leukemia virus (BLV). The major differences between EIAV and type C virions are the absence of the inner membrane characteristic of type C viruses, and the presence of an intraviral membranous tubule in addition to the electron dense core in mature extracellular EIAV. Negative staining showed EIAV to have head-tail shapes characteristic of type C virus. Bovine leukemia virus had previously been described as a retravirus with the morphology of type C viruses. Biochemically BLV more closely resembles mouse mammary tumor virus (MMTV) and Mason-Pfizer monkey virus (MPMV). Microscopic examination of BLV infected sheep cells contain two morphologically distinguishable particles within individual cells. One particle resembles the type C virus while the other resembles MPMV. It was concluded that the particles budding under the plasma membrane and resembling type C virions, are only phenotypic variants of RLV. A similar situation exists for MMTV.

Significance to Biomedical Research and the Program of the Institute: Major emphasis has been placed on the development and application of the scanning EM and associated energy dispersion X-ray system to viral oncology studies. New preparation methods developed for visualization in the SEM will contribute an additional highly specific mapping system to the fields of immunology and virology.

Proposed Course of Project:

Further development of the energy dispersive X-ray system and its application to immunological tagging will be pursued. Emphasis will be placed on continuing the development of specimen preparation and instrumentation as it applies to the ultimate goal of the program.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04990-03-ADVO

PERIOD COVERED
July 1, 1976 to Sept.30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation and Expression of Mammalian RNA Tumor Viruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R.J. Goldberg Microbiologist ADVO NCI

Other: None

Cooperating Units

Drs. Edward Scolnick, Dante Marciani, Steven O'Brien, Takis Papas, and John Dahlberg, VOP,DCCP,NCI
Dr. John Docherty, Pennsylvania State University, College Park, PA.
Dr. Richard Crowell, Hahnemman Medical College, Philadelphia, PA.
Dr. David Kingsbury, University of California, Irvine, CA.

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program

SECTION

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.0	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒(b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The rat cellular sequences homologous to two murine sarcoma viruses have been studied in respect to their biochemical behavior and biological function. Studies have been initiated to define the role of type C virus expression on myogenic differentiation in cultured mammalian cells. Several mutant mammalian cell lines have been derived and characterized. Somatic cell hybrid cell lines have been studied in terms of correlating RNA tumor virus expression as a function of phenotypic behavior. Experiments have been conducted to define the interaction between RNA tumor viruses and human herpesviruses.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

(1) To characterize type C virus expression in differentiating cultured cells. (2) To study the interaction between RNA and DNA tumor viruses. (3) To study the genetic regulation of RNA tumor virus expression. (4) To define the biological function of endogenous sarcoma-specific RNA tumor virus genetic sequences. (5) To isolate and characterize new RNA tumor viruses.

Methods Employed:

Various tissue culture assays and procedures have been employed including: cloning of cells in soft agar and microtest II plates, focus-assays for type C helper viruses, somatic cell hybridization, co-cultivation procedures, infectious center assays, establishment of primary cell cultures from a variety of animal tissues, and the induction and propagation of a variety of RNA tumor viruses in cultured cells.

Biochemical methods used during the last year have included isolation and purification of viral and cellular nucleic acids, the use of s1 nuclease for hybridization assays, the synthesis of DNA transcripts by use of the endogenous reaction of RNA tumor viruses, subcellular fractionation procedures, the separation and characterization of various classes of RNA, and the identification and quantitation of mammalian cell enzymes.

Major Findings:

RNA sequences in cultured rat cells homologous to the rat specific information in the Kirsten and Harvey strains of murine sarcoma viruses have been characterized. These sequences have been shown to share several characteristics with those of replicating type C viruses including their rescuability, size, and inducibility with halogenated pyrimidines. Although all rat cell lines examined have multiple copies of sarc-specific sequences in their DNA, expression at the RNA level is quite variable. Somatic cell hybrids of mouse-rat origin have been shown to express sarc-specific RNA.

Studies were undertaken to determine whether differentiating cultures of a myogenic rat cell line spontaneously released endogenous type C virus and if expression of such a virus was linked to cell differentiation. The fusion of mononucleated myoblasts into multinucleated myotubes was used as a marker of differentiation and virus expression was determined by detection of particle-associated RT activity in cell culture fluids. Initial results indicate that release of endogenous rat type C virus occurred in cells programmed to differentiate and that cell fusion was not a requirement for virus expression. The effect of exogenous infection with a variety of type C viruses on myogenic differentiation has also been studied. Viruses have been preliminarily categorized in respect to their effects on *in vitro* myogenesis at the cellular and molecular level.

Studies have been continued on the factors regulating RNA tumor virus expression by somatic cell hybridization. Additional somatic mutants of various mammalian cells have been derived by plating cells in media designed to select variants with known genetic markers. Somatic cell hybrids have been derived from these mutant cells are now being studied to map the location and regulation of various type C and type B virus functions.

Rat cells expressing various levels of endogenous type C information have been characterized in terms of their susceptibility to herpes simplex virus types 1 and 2. A spectrum of susceptibilities has been described. Studies are in progress to correlate various cell markers, including endogenous type C virus expression, with herpes susceptibility. A type C virus has been co-cultivated in mink cells from human lymphoblastoid EBV-positive cells which had been passaged through nude mice. The involvement of EBV in virus rescue in this system is being investigated. The ability of HSV to activate type C viral and cellular (non-viral) genes is under investigation. A convenient murine system with defined viral and cellular markers has been chosen for study. Selection techniques for the derivation of HSV transformed cells have been developed.

Significance to Biomedical Research and the Program of the Institute:

Oncornaviruses have now been isolated from a variety of mammalian and avian species. Many of these viruses have been shown to be involved etiologically in the induction of malignant diseases. Although type C or type B viruses have not been isolated from human cells, subviral footprints have been detected in various human tissues. In addition, type C viruses from several mammalian species have been shown to replicate in and, in some cases, transform human cells. Therefore, it is obvious that these self-contained packages of genes offer a convenient and relevant means to study the mechanism of cell transformation. It is also imperative that the search for a bona fide human tumor virus be pressed. Ultimately this would facilitate a thorough study of the involvement of this class of viruses in human neoplasia and open a rational approach for immunoprophylaxis and therapy of these diseases.

Proposed Course:

Studies planned for the next year will concentrate on characterizing type C virus expression following cell transformation by mammalian RNA and DNA viruses. Particular attention will be directed at studying RNA tumor virus expression in cells which have been morphologically or biochemically transformed by various human herpesviruses. Attempts will continue to isolate and characterize RNA tumor viruses and to detect virus information in various mammalian species, especially higher primates including man. The program to study RNA tumor virus expression as a function of cell differentiation will be expanded. Genetic studies dealing with the chromosomal location of tumor viruses and the regulation of their expression will be continued.

Publications:

Scolnick, E.M., Goldberg, R.J., and Williams, D.: characterization of rat genetic sequences of Kirsten sarcoma virus: a distinct class of endogenous rat type C virus. J. Virol. 18: 559-566, 1976

Scolnick, E.M., Williams, D., Maryak, J., Vass, N., Goldberg, R.J., and Parks, W.P.: Type C particle-positive and type C particle-negative rat cell lines: characterization of the coding capacity of endogenous sarcoma virus-specific RNA, J. Virol. 20: 570-582, 1976

Bendas, C.M., Crowell, R.L. and Goldberg, R.J.: The spontaneous release of endogenous rat type C virus from myogenic cells in culture. In Vtiro 13: 172, 1977

d. Editorial Unit

The Editorial Unit within the Office of the Associate Director performs a variety of special services for the entire Viral Oncology Program. It is primarily responsible for the collection and dissemination of scientific information in the area of virus cancer research. Services are available to all staff members, Virus Cancer Program contractors and, as far as possible, to persons outside the Viral Oncology area. During the past year, major activities have included the following:

(1) Program Analysis System: The Editorial Unit has responsibility for the technical operation of the Viral Oncology Program Analysis System which provides scientific, administrative and fiscal information to aid in the planning, monitoring and evaluation of the collaborative program.

Current system reports (project indices to record key, contractor and contract number; and a Section Project Summary which displays funding and date information) are produced monthly for use by Section Head and other scientific and administrative personnel including contract specialists. Committee Review Forms are generated as needed during the normal project review cycle.

Scheduling and tracking reports providing "calculated" and "scheduled dates" are produced both as periodic and demand reports. Information is processed for new competitive contracts, new sole-source and non-competitive contracts with incremental funding and all contract renewals.

Retrievable information on Viral Oncology projects is contained in two files: (a) the Project File, which contains funding, scheduling, current status and other administrative information; and (b) the Abstract File, which contains scientific descriptions of projects in the form of keyword descriptors and abstracts. Through use of a query subsystem, the data base can be searched and specific information provided to an individual user in a short period of time. The staff of the Editorial Unit is available to assist in planning queries to extract the information required in the most usable format.

(2) Contractors' Progress Reports: Annual and semi-annual reports of scientific progress are collected from Virus Cancer Program contractors for distribution to program management personnel and review committees. A final report covering the whole period of performance is requested when a contract terminates.

(3) Summaries of Progress Reports: A booklet containing summaries of progress reports by Section is published in February and August for distribution to key program scientists. The booklet is intended to be used as a convenient reference to current VCP research.

(4) Virus Cancer Program Bibliography: The Editorial Unit prepares a comprehensive bibliography which includes all papers published by Viral Oncology staff members and by investigators under contracts and

grants sponsored by the Virus Cancer Program. This bibliography is used in the NCI Annual Report and the Annual Progress Report.

(5) Collaborative Directory: This directory includes data on all VCP contracts and grants including a listing of principal investigators, institutions, project officers, contract specialists and Section or Office managing each project. It is published and updated at appropriate intervals and is designed to facilitate communications between staff members and contractors.

(6) Viral Oncology Library: The Editorial Unit is responsible for maintaining a small library which contains 400 volumes including basic reference texts, monographs and over forty professional journals specializing in cancer research. It is primarily for use by laboratory scientists in Building 37.

2. a. LABORATORY OF DNA TUMOR VIRUSES
July 1, 1976 - September 30, 1977

The Laboratory of DNA Tumor Viruses plans and conducts research on viruses with a DNA core to define their role in the development of cancers in animals and man; develops and applies biological, biochemical and immunological procedures to obtain evidence for virus genetic expression in neoplastic cells; and investigates the mechanisms by which cellular gene expression, viral gene expression, and the interactions between viruses influence transformation of cells.

The Microbiology Section applies virologic, biochemical and immunological techniques to the study of the biology of herpesviruses and the nature of their association with certain cancers in humans and in animals with emphasis on the elucidation of the mechanisms whereby viruses of this group establish occult infections in cells which terminate in neoplasia; and defines the role of herpesviruses in oncogenesis by investigation of their effect on normal cellular control mechanisms and of the effects of herpesvirus and type C RNA virus interactions in dually infected cells. The Virus Tumor Biology Section studies the mechanisms of cellular transformation induced by human and simian papovaviruses; evaluates the effects of viral infection on cellular control mechanisms by comparing transcription and translation of virus genome information in transformation and in cytolytic responses to infection; and studies specific portions of the viral genetic sequences responsible for cellular transformation by genetic mapping of the small DNA viruses. The Virus Tumor Biochemistry Section conducts research on the specific protein and nucleic acid components of DNA viruses, and includes capability for the isolation and characterization of virion structural and nonstructural proteins of RNA viruses for collaborative studies on virus interactions in the oncogenic process; studies translational processes in vitro; and maintains electron microscope support for the research conducted by the laboratory. The Cell Physiology Section studies the alterations which occur in cells transformed by various means, including DNA tumor viuses; identifies enzymes of viral or cellular nature and determines the effects of transformation on cellular enzymes; investigates the influence of virus infection on the regulation of cellular enzymes with emphasis on the delineation of processes which result in repression or derepression of normal cell components; studies changes in the properties of cellular membranes induced by virus infection and their relationship to malignancy; and provides expertise for the study of both RNA and DNA viruses to complement activities within the Laboratory. The Primate Virus Section studies the relationships between virus and host factors that contribute to the pathologic processes involved in oncogenesis with particular emphasis on herpesviruses associated with induced neoplasia and cellular susceptibility genes that may predispose individuals to the development of cancer. The Office of the Chief coordinates the research conducted in the Sections with due recognition to the scientific freedom of the individual investigators. The Office is responsible for establishing collaborative research efforts between investigators in the Laboratory and other laboratories at NIH or elsewhere. The Chief directs the general activities within the Laboratory with the aid of the Assistant Chief.

The Primate Virus Section was dissolved during the past year with reassignment of its personnel component to other duties. The Virus Tumor Biology Section and the Cell Physiology Section were staffed and equipped, and active programs of research were begun. The staff of the Microbiology Section was relocated at the Frederick Cancer Research Center. The Head of the Primate Virus Section was invited by the Director, Institute of Medical Microbiology and WHO Collaborating Center for Comparative Virology, University of Munich, West Germany, to be a consultant to the Center on a regular basis. He has been active in international cooperation and coordination of investigations on oncogenic herpesviruses. As a reference laboratory for the WHO/FAO Program on Comparative Virology and for the IARC International Reference Program for Oncogenic Herpesviruses (IRP/OHV), the Primate Virus Section participated in serological testing of human and simian specimens for reactivity against different herpesviruses. It also provided reference virus strains and reference sera for use by other investigators.

Comparative serology was conducted to evaluate the humoral immune responses against four Epstein Barr virus (EBV) antigens of patients with nasopharyngeal carcinoma (NPC) or Burkitt's lymphoma (BL) residing in different geographic areas of the world. In general, NPC patients in Tunisia and Hong Kong, areas with a moderate to high incidence of this cancer, developed higher antibody titers than did NPC patients in the United States. Similarly, African BL cases had higher antibody levels to EBV antigens than did American patients with a diagnosis of BL. American NPC patients with Chinese surnames developed antibody titers comparable to those in Hong Kong Chinese, suggesting genetic predisposition.

A joint study was conducted with investigators at the Pathology Institute, Cologne, to determine the prognostic significance of immunocytological, cell culture and serological findings on specimens from patients with malignant lymphoma. Significant serum titers against EBV antigens usually were noted in association with tumors with lympho-plasmacytoid differentiation. Cells of these tumors proliferated actively in vitro. The patients responded well to initial therapy. Tumors from patients with no significant antibody titers to EBV generally grew slowly in culture, and there was marked to rapid progression of the disease. Among these tumors, lymphomas with B-cell markers usually had a more favorable clinical course in terms of therapy and progression of disease.

Herpesvirus saimiri is horizontally transmitted between squirrel monkeys, the natural host species, but produces no overt disease. Owl monkeys, Aotus trivirgatus, have been observed to acquire infection by this virus through contact with squirrel monkeys. These animals subsequently developed lymphoma. However, the virus was not transmitted horizontally from infected owl monkeys to cagemates. This may be ascribed to the apparent inability of owl monkeys to secrete cell-free virus by the oropharyngeal route.

The influence of the host and of the virus-carrying cell on the virus-cell relationship was demonstrated by implantation into nude mice of cells of the EBV-carrying HR1 line of BL cells and of somatic cell hybrids of HR1 with D98 cells. Both the HR1 and the HR1/D98 hybrid cells grew to produce tumors in these mice. A considerable reduction in the amount of viral DNA

carried by these tumor cells was observed following their passage in vivo. Furthermore, the HR1 cell tumors from nude mice were observed to contain a type C RNA virus budding from cell membranes, apparently a xenotropic virus activated in the nude mice bearing the tumor and replicating in the tumor cells. On the other hand, the HR1/D98 hybrid cell tumors showed no evidence of any active virus production.

Herpes simplex viruses (HSV) are widely disseminated in human populations. Type 1 strains are commonly associated with the development of oral lesions, type 2 strains with genital lesions. These viruses establish persisting covert infections which may give rise to recurrent overt disease. Particular attention has been given to the association of type 2 strains with carcinoma of the uterine cervix. Investigations to ascertain the role of HSV-2 in this neoplasm in humans were pursued in experimental animal cell systems. Since the virus destroys cultured cells, ultraviolet (UV) light irradiation of HSV-2 was used to inactivate its cytolytic property. Such irradiated virus (UV-HSV) did induce a malignant transformation of cultured hamster and mouse cells. Since these animals carry endogenous type C RNA viruses, investigations were initiated to determine whether the HSV was the primary cause of the transformation, whether it was a co-factor in the neoplastic process, or whether it was a non-specific activator of a series of intracellular events involving the endogenous type C virus as the primary etiologic agent.

Previous studies which demonstrated the ability of UV-HSV to activate type C virus were conducted in BALB/c mouse cells which carried a defective mouse sarcoma virus genome. The activation of the endogenous type C virus by UV-HSV resulted in the release of virions containing the sarcoma virus genome which was detected by the production of infectious centers on a culture of permissive rat cells. These studies have now been extended to sarcoma genome-negative BALB/c mouse cells as a preliminary to correlating type C virus activation with transformation by UV-HSV. In this system, activated xenotropic type C virus was detected by focus formation in the F81 line of cat cells. Type C virus activation occurred at a maximum frequency of $1\text{-}3 \times 10^{-4}$ depending on the UV dose and the multiplicity of infection.

The BLP line of BALB/c mouse cells was found to yield reproducible results in transformation assays and has been selected for further studies. Transformed cell colonies showed variability in their degree of loss of contact inhibition and cell morphology, suggesting that transformation by UV-USV is a multi-step process. Parallel studies with chemical carcinogens show similar variations in properties of transformed cells. BLP cells transformed by UV-HSV or by chemical acquire the ability to grow first in agarose and then in agar. Detection of transformed cells by the acquired capability to grow in agarose was found to be more sensitive and less time-consuming than awaiting appearance of transformed cell foci. Transformation by UV-HSV appears to be correlated with type C virus activation in that cultures which yielded transformants after 6 to 10 weeks of cultivation also showed type C virus activation on the first day after infection with UV-HSV. A direct cause and effect relationship remains to be established. However, present data suggests that HSV may act as a co-carcinogen by altering expression of type C virus genetic information.

The possibility was considered that activation of endogenous type C virus genetic expression by UV-HSV might occur in many more cells than was detected by infectious center formation in indicator cells. Accordingly, an immunofluorescence assay procedure was applied to detect any increased synthesis of the p30 type C viral polypeptide in treated cultures. A rabbit antiserum, monospecific for the p30 antigen, was used without success because it was observed that rabbit sera could stain HSV infected cells non-specifically. However, substitution with a radioimmunoassay procedure was satisfactory. This showed no increase in p30 antigen production after cells were infected with HSV, indicating that type C virus activation was not induced in significantly more cells than was detected by infectious center formation.

Two non-transformed cell lines were established for study of the cell physiology of the transformed state, the BLP line and the muntjac line. The BLP line of BALB/c mouse embryo cells was cloned, and several sublines were established which meet the criteria of non-transformed cells, namely, contact inhibition, anchorage dependency and non-tumorigenicity in BALB/c mice. A stable non-transformed line of muntjac cells, established from Indian deer and a cloned line of transformed muntjac cells, tumorigenic in nude mice, both possess 7 chromosomes. These muntjac cell lines may provide opportunity to study oncogenic mechanisms at the chromosomal level.

The uptake of sugars increases during and after cell transformation. Transition of the sugar uptake system from a saturable to a nonsaturable process occurs near the physiological concentration of D-glucose normally seen in animal blood. At concentrations higher than $5x10^{-3}$ M sugar, where a saturable process is barely involved, nonsaturable uptakes of D-glucose, D-mannose, D-galactose, 2-deoxy-D-glucose and 3-O-methyl-D-glucose proceed ten-to hundreds-faster than the rate of simple diffusion of L-glucose. The nonsaturable uptake may be part of the total physiological process which, along with the saturable process, is controlled by a membrane coordination system.

The persistent production of type C RNA tumor viruses appears to be related to the number of gene copies of proviral DNA in the cell. This was observed by treatment with 5-BUdR of a nonproducer BALB/c mouse 3T3 clone transformed by Kirsten murine sarcoma virus. Subclones were established; each was characterized for its differential expression of viral RNA and analyzed with DNA complementary to Kirsten sarcoma virus RNA by hybridization kinetics. All subclones contained nearly full complements of viral DNA, but the copy numbers were higher in persistently shedding lines as compared to non-producing lines.

Members of the papovavirus group of small DNA viruses have been shown to be oncogenic in laboratory animals. Two members of this group, simian virus 40 (SV40) and the BK virus (BKV) isolated from humans are under study in this Laboratory. Nucleic acid hybridization analyses, using viral DNA probes, were applied to search for the presence of viral genetic information within human tumors and in cultured tumor cells. To date, no evidence of the presence of BK or SV40 copies were detected in these tissues. To determine whether BKV could transform human cells, the cytolytic function

genetic complement of viral information. These defective viral genomes occur presumably from deletions and recombination of genetic information. Transforming viruses of this type are the Group C adenoviruses, murine sarcoma-RNA tumor viruses and perhaps the herpes viruses. In the latter case defective genomes of Herpesvirus saimiri (HVS) are characteristically found in both virions and in transformed cells. The latter two classes of viruses have been studied to determine the mechanism of defective genome generation, and the products they express.

Herpesvirus saimiri infection of owl monkey kidney cells induces the appearance of a unique nuclear DNA polymerase. This polymerase can be distinguished from cellular polymerases by its high salt requirement and its lower sensitivity to changes in temperature and pH. It has been purified through two column procedures and has a molecular weight of about 190,000 daltons. The enzyme has a preferential template specificity for high G+C and appears to be insensitive to phosphonoacetic acid (PAA). The former property may be related to the selection and maintenance of the high G+C defective HVS genomes generated from the ends of the infectious HVS DNA. The lack of enzyme PAA sensitivity is curious since HVS replication in vivo and in vitro is sensitive to the drug and the polymerase induced by other herpesvirus was markedly inhibited by PAA. The HVS induced DNA polymerase is produced in the presence of PAA and this could suggest that some other step in HVS replication may be sensitive to PAA.

The origin of herpes simplex virus type 1 (HSV-1) defective genomes (dHSV-1) in the infectious viral DNA has been physically mapped with endonuclease restriction (endo R) enzyme Bam HI. This study demonstrated that the repeating 5.5-5.7 megadalton dHSV-1 DNA arises between the third and fourth Bam HI (B_3, B_4) cleavage sites (4.7 and 5.8 megadaltons respectively) from the right (end) 8 megadalton Hind III G fragment. The heterogeneity observed in the dHSV-1 DNA (about 0.3 megadaltons) is located in the recombinational junction between the normal right end of HSV-1 DNA and the new-premature termination which occurs between B_3, and B_4 sites. This could result from heterogeneity in the ends of HSV-1 DNA replication and will be an excellent system for studying the generation of deletions and recombinations in viral genes.

Three methods have been developed which will facilitate working with HSV. Small DNA fragments are required to study HSV DNA in a conventional manner: DNA sequencing, origins of transcription, and product expression. It has been technically difficult to map HSV DNA with R enzymes that extensively cleave the DNA due to its 100 megadalton complexity and its multiple forms inverted about an internal repetition. A modification of the blotting technique is being developed which should greatly facilitate obtaining endo R linear maps of extensively cleaved HSV DNA. This procedure consists of blotting unlabeled endo R cleaved DNA in one direction followed by blotting to the same filter at right angles under hybridization conditions with isotopically labeled endo R cleaved DNA. In this manner, endo R fragments

of the virus was inactivated by ultraviolet irradiation, and the irradiated virus was applied to human embryonic kidney cells. Several putative transformed human cell lines were isolated and are being further characterized.

The SV40 and BKV genomes were shown to share at least 20 percent sequence homology, a clear indication that they have a common evolutionary ancestor. A non-stringent electron microscopic technique discolsed that over 50 percent of the genomes share looser homology with considerable mismatch. However, the genome segments containing the viral initiation sites for DNA replication remain heterologous. Possibly this sequence, which interacts directly with the host cell DNA polymerase, may be crucial in determining the host range of the viruses. Preliminary results of sequencing the BKV initiation site for comparison with SV40 suggest that the two segments, while sequentially distinct, share a very similar topology.

The T-antigen in SV40 induced tumor and in lytically infected cells is coded by about one-half of the virus genome corresponding to the early region as defined by transcriptional mapping. The other half of the genome, the late region, codes for viral structural proteins. The mode of late transcription was shown to depend on a functional T-antigen, and the amount of late viral transcription is directly related to the amount of preceding viral DNA replication. Studies on the interactions between papovaviruses showed that the BKV early function, presumably T-antigen, permits a DNA-negative early SV40 ts mutant to replicate its DNA at the non-permissive temperature.

The intracellular synthesis of nascent SV40 RNA molecules is being investigated to evaluate in a temporal way the sequence of nucleic acid processing events, including cleavage from precursors, methylation and capping, and polyadenylation. The entire late strand of viral RNA is synthesized to unit length at late stages in the lytic cycle. The processed products are being mapped. Deletion mutants of the virus are being used in attempts to define the control regions for RNA synthesis and processing.

Investigations were directed to the mechanisms of integration of papovavirus DNA sequences within host DNA and the effect of this integration on normal viral or host-cell mechanisms. It was found that a defective virus genome which contains host cell DNA sequences produces no host cell-specific RNA *in vivo* although host-specific transcription could be demonstrated *in vitro*. This suggests that these RNA sequences are synthesized in cells, but for some reason, are rapidly degraded. The factors responsible for degradation of the hybrid mRNAs are being investigated and attempts are being made to determine in which set of sequences the promoter lies.

Research emphasis has been placed on the specific protein and nucleic acid components of RNA and DNA tumor viruses. One of the common features of the different viral transformation systems that have been studied is that only certain portions of the viral genome are expressed in transformed cells and is especially apparent with several transforming viruses that lack a full

generated with one enzyme can be directly related to fragments generated with a second enzyme. This method will be used to compare the linear genomic arrangements of HSV-1 and HSV-2

A method has been developed to enrich for and isolate cellular RNA sequences homologous to HSV DNA. Single-stranded HSV DNA covalently linked to Sepharose can, under hybridization conditions, be used to isolate HSV specific cell RNA sequences in a 10,000 fold excess of normal cell RNA. This HSV specific RNA can be subsequently isolated from the Sepharose by elution with formamide. This technique will be useful for isolating cellular RNA homologous to specific HSV DNA fragments, and especially for isolating RNA from HSV transformed cell.

The third method developed is a modification of conventional transfection assays and utilizes a DNA transfected cell layer (primary) to transfect indicator cells (secondary). The assay is quantitative and reproducible and can increase the sensitivity of the primary assay 20 fold. With this technique plaque titers of HSV DNA have been obtained as high as 2,000 per μg. The obvious advantage of this technique is that the primary-transfected cell layer can be transferred to many different heterologous secondary cells and can be used to screen cell susceptibility to transfection or more importantly, to transformation.

The gag polyprotein, P60, specified by the defective Moloney murine sarcoma virus genome has been extensively characterized. Antibody prepared to the purified protein was shown to recognize determinants on Moloney murine leukemia virus (M-MuLV) p30, p15, and p12. In complement-dependent assays the anti-P60 serum was shown to neutralize heterologous RNA tumor viruses and to cause virolysis. This demonstrated that in addition to gp71 and p15E, interspecies gag protein determinants were on the surface of virions. The anti-P60 serum was used to demonstrate that P60 is present in all mlMSV transformed cells and that the cellular polyprotein is stable to cleavage. The site of synthesis and the location of P60 in both producer and non-producer mlMSV transformed cells in pulse-chase experiments showed that the polyprotein was synthesized *in vivo* and *in vitro* in a low speed sedimenting membrane fraction. P60 was also shown to appear in the *in vivo* chase in a high salt extractable nuclear fraction.

By peptide analyses the virion mlMSV P60 was shown to contain the p30, p15, and p12 virion cleavage products. P60 was also shown to be phosphorylated in both virus and in producer and non-producer transformed cells. Of the three cleaved products of P60 only the p12 was phosphorylated and both polypeptides were phosphorylated in threonine and serine residues. This suggested that P60 phosphorylation occurs specifically in the p12 moiety. Both virion and cellular P60 were retained to single-strand DNA Sepharose columns and could be eluted with 0.15 to 0.6 M KCl. This property was previously shown to be a characteristic of the basic RNA tumor virus protein p10. However, no p10 peptides were detected in P60 and suggested that the P60 binding was due to either the conformation of the polyprotein or to one of the constituent polypeptides such as p12. The phosphorylation and

nucleic acid binding properties of P60 are characteristics of regulatory proteins such as the histone and non-histone chromosomal proteins. Thus the gag polyprotein could be involved in cellular transformation perhaps by regulating the expression of specific cellular sequences.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04834-08-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Epstein-Barr Virus, Herpesvirus Saimiri, and Herpesvirus Ateles

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. V. Ablashi	Head, Primate Virus Section	LDTV NCI
OTHER:	J. M. Easton	Medical Director	LDTV NCI
	P. H. Levine	Senior Surgeon	LVC NCI
	J. Luetzeler		LVC NCI

COOPERATING UNITS

G. Pearson	Dept. Microbiology, Mayo Clinic, Rochester, MN
R. Glaser	Dept. Microbiology, M. S. Hershey Med. Ctr., Hershey, PA
M. Nonoyama	Life Sciences, Inc., St. Petersburg, FL
D. Stevens	Dept. Medicine, Santa Clara Valley Med. Ctr., San Jose, CA
G. de Thé	International Agency for Research on Cancer, Lyon, France
R. Krüger	Pathology Inst., Univ. of Cologne, West Germany
M. Shah	Litton Bionetics, Inc., Frederick, MD
H. Rabin	Litton Bionetics, Inc., Frederick, MD

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Primate Virus Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The oncogenic potentials of Epstein-Barr virus (EBV) in nonhuman primates and the continuous demonstration of EBV genome in epithelial cells in nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL) provide strong evidence that this virus is one of the major contributing factors in the complex etiology of these tumors. Moreover, the oncogenicity of Herpesvirus saimiri (HVS) and Herpesvirus ateles (HVA) in nonhuman primates and in certain rodents relates oncogenic herpesvirus to lymphoproliferative neoplasia.

The international activities consisting of coordination between NCI Viral Oncology Program and W.H.O. International Reference Programs in Comparative Virology were continued. Under this activity we provided consultation, exchange of scientific data, personnel, reference reagents and collected data on comparative oncology from different parts of the world to relate to etiological factors in tumors from man and domestic animals.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To study herpesviruses associated with neoplasia in humans and non-human primates to obtain better understanding of the host-virus relationship with respect to disease.

B. Participation in and initiation of international collaborative activities of mutual interest to NCI, Viral Oncology Program, and W.H.O. International Reference Program in Comparative Virology.

Methods Employed:

Primary cell cultures and continuous cell lines of human and animal origin were used for virus isolation, biological and biochemical assays, and *in vivo* experimentation. Standard biochemical and immunological procedures were applied.

Major Findings:

The Primate Virus Section studies the interaction and clinical implications of tumor viruses of human and subhuman primates by employing immunovirological, biochemical and pathological assays. At present a greater emphasis has been placed on human Epstein-Barr virus (EBV), oncogenic *Herpesvirus saimiri* (HVS) of squirrel monkeys and *Herpesvirus ateles* (HVA) of spider monkeys. The section also participates as an international reference laboratory for two World Health Organization programs on comparative virology.

The incidences of squamous cell carcinoma of the nasopharynx (NPC) and of Burkitt's lymphoma (BL) are relatively high in some parts of the world, and moderate to low in other places. A study was conducted to evaluate the serologic response to EBV patients with nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL) from Hong Kong, Tunisia, North America, France, West Germany and Africa in order to find whether viral relationships in these diseases are the same in different geographic areas and to determine those factors which are etiologically important. In general the antibody titers to four EBV antigens were highest in NPC patients from high and moderate incidence areas, e.g. Hong Kong and Tunisia. With one exception, titers were not elevated in the German NPC patients. The titers were intermediate in patients from the U.S.A. and France. NPC patients with Chinese surnames in the U.S.A. had comparable titers to the Hong Kong group. BL patients from Africa had higher anti-EBV titers than patients from the U.S.A. Virus capsid antigen (VCA) titers in American BL patients appeared to be either very high or very low, suggesting two distinct groups relating to EBV but not to age or mode of disease presentation. These data on a limited number of NPC and BL sera suggest genetic predisposition and pathologically similar NPC.

In collaborative studies with the Mayo Clinic, immunological responses to EBV were studied on American NPC patients in order to find any clinical application. High antibody titers to EBV early antigen (EA) and the presence

of antibodies to the EBV-associated antigen in the IgA immunoglobulin fraction were the two parameters that frequently distinguished NPC patients from those with other malignancies. NPC patients undergoing therapy could be followed successfully by a cytotoxicity assay measuring antibody to EBV membrane antigen.

A study was jointly conducted with the Pathology Institute, Cologne, to investigate the prognostic significance of immunocytological, tissue culture and serological studies in patients with malignant lymphoma (ML). About 75% of ML were of the B-cell type. Elevated anti-EBV titers were observed primarily in sera from cases with well-differentiated ML and Burkitt's type lymphoma. Of the well-differentiated ML, significant anti-EBV titers were usually noted in tumors with lympho-plasmacytoid differentiation. These tumors proliferated actively in vitro and generally responded well to initial therapy. Disease progression was rather slow in EBV-positive MLW, while it was quite progressive in Burkitt's type lymphoma. Tumors with no significant anti-EBV titers were variable. Generally these were slow-growing in cell culture and disease progression was marked to rapid. Among these tumors, lymphomas with B-cell markers usually had a more favorable clinical course in terms of therapy and disease progression.

Cell-mediated immunity (CMI) to EBV was investigated in infectious mononucleosis (IM) patients, EBV-seronegative and seropositive healthy donors and patients with other acute viral infections. The assays employed were phytohemagglutinin (PHA), EBV-VCA (purified virus and culture supernatant), soluble antigen-induced blast transformation of purified mononuclear cells in autologous and EBV antibody-negative AB serum and the production of the lymphokine lymphotoxin. The data demonstrated specific CMI to EBV. They indicated that CMI develops slowly in IM and contracts with the evolution of clinical events and humoral immunity. CMI could thus be the mechanism of terminating lymphoproliferation in IM. IM patients have transient serum factor blocking transformation.

Herpesvirus saimiri has been shown to be horizontally transmitted between squirrel monkeys, the natural host species. It has also been transmitted to owl monkeys (Aotus trivirgatus) through contact with squirrel monkeys. The horizontal transmission from HVS-inoculated owl monkeys to uninoculated owl monkeys in the same cage or room has not been reported. No cell-free HVS was detected in the oropharyngeal secretions of HVS-inoculated owl monkeys developing leukemia or lymphoma. These animals failed to transmit either virus or disease to their uninoculated cagemates or roommates. This may be due to the apparent inability of owl monkeys to produce cell-free virus or to secrete it by the oropharyngeal route. The results could not only be useful in formulating plans concerning housing of owl monkeys for HVS studies but may also provide insight as to how human herpesviruses are maintained in the oropharynx.

B95-8 EBV has the ability to transform lymphocytes of human and nonhuman origin and it has been suggested that donors' age could also affect the transformation by EBV and virus expression. Transformation occurred 14-34 days post-infection in cells from newborn or young animals and 35-40 days

post-infection in cells from 2 year old adults. Perhaps the delay in transformation could be due to changes in cell receptors to EBV as the animals get older and this may also affect the cellular control of viral expression in the transformed cells.

Previously we reported the induction of undifferentiated carcinoma and poorly differentiated lymphoma in athymic nude mice by inoculating EBV-carrying-HRI and HRI-D98 somatic cell hybrids respectively. Due to limited availability of nonhuman primates for EBV studies, nude mouse tumors were found to be inexpensive models for studying the regulation of the EBV genome in epithelial and lymphoid cells as well as for evaluating antiviral and antitumor drugs. Further data suggested that both types of tumors were transplantable in nude mice. Their induction was found to be consistent. After a few serial passages in vitro and in vivo there was enhancement in tumor incidence and size. HR-I cell-induced tumors always appeared approximately 30-40 days post-inoculation and the incidence rate ranged between 55-60%. EBV hybridization data on tumors and tumor cells in culture showed that the reduction of EBV-DNA was about 300-fold in HR-I-induced tumors and established cell lines. A ten-fold reduction was found in D98/HR-I tumors and established cell cultures. The reduction of EBV genomes in the tumor cells suggests that in vivo passage can influence the number of EBV genomes in these cells. Greater reduction with the HRI system was due to the fact that EBV production was stopped in vivo. Early cell cultures of both HR-I or D98/HR-I-induced tumors failed to provide evidence of type C virus expression; however, in the ninth passage of HR-I, type C budding particles were observed. So far no virus of any sort has been observed in D98/HR-I established cultures. Since the chromosome analysis indicates that all the above cultures are of human origin, it is likely that these type C particles may represent a xenotropic virus of nude mice or another virus possibly associated with HR-I cells.

The members of the Primate Virus Section presented five papers at the National and International scientific meetings.

The Section Head participated as a faculty member in teaching the post-graduate International Course in Immuno-Virology of Cancer sponsored by the World Health Organization International Agency for Research on Cancer (WHO I.A.R.C.) in Lyon, France (May 10-21, 1976). He also served as consultant to the Veterinary Public Health Unit of WHO in Geneva, Switzerland (May 23-26, 1976). The Section Head was invited by the Director, Institute of Medical Microbiology and WHO Collaborating Center for Comparative Virology, University of Munich, West Germany, to be consultant on a regular basis to the Center. He was a principal reviewer and discussant on oncogenic DNA virus session of the International Symposium on Experimental Animals and In Vitro Systems in Medical Microbiology held at the WHO Center, Munich (September 14-16, 1976). He was also invited by the Danish Association for Cancer Research to deliver a lecture on cancer and oncogenic herpesviruses of man and subhuman primates at the Cancer Hospital and Institute of Microbiology, University of Copenhagen, Denmark (September 24, 1976).

The Primate Virus Section, as a reference laboratory for WHO/FAO Program on Comparative Virology and for the IARC International Reference Program for Oncogenic Herpesviruses (IRP/PHV), participated in testing human and simian sera against EBV, HVS, HVA and human and simian cytomegaloviruses. It also supplied, through the International Reference Program, reference virus strains and reference sera for HVS and HVA to investigators. The laboratory also prepared a comprehensive list of references (papers published, abstracts and papers in press) on oncogenic herpesviruses of old and new world primates for the IRP/OHV.

Significance to Biomedical Research and the Program of the Institute:

A. The findings in A.1-7 on EBV, HVS and HVS related to clinical and laboratory studies confirm, extend and initiate approaches in biochemical, immunovirological and pathological investigations in determining cell-virus interaction and immunological parameters which might provide valuable clinical tools for the diagnosis, prognosis and better control of certain neoplasms where a herpesvirus is considered a major contributing etiological factor. The response to EBV in NPC and BL cases from different geographic locations suggests genetic predisposition and that NPC is pathologically identical in these areas. There may be immunologically different entities as is also suggested in BL. Within a certain population, genetic predisposition and environmental factors could be major contributing factors to the etiology of NPC and BL and may also be useful in the identification of individuals at risk. The lack of a horizontal transmission of HSV in epxerimentally infected owl monkeys may provide insight as to how human herpesviruses are maintained in the oropharynx. Some of the findings described here not only suggest an independent contribution of a virus in certain neoplastic diseases, but also relates to the host factors which may affect the outcome of viral infection, cell transformation and tumor induction.

B. The international collaborations have significantly contributed to the Program because they have stimulated exchange of information, dissemination of standard reagents, and comparison, discussion and teaching of techniques in immunovirology of cancer. Emphasis in certain areas of research through the two international reference programs has provided investigators identification of sources for specific materials and has also aided in avoiding unnecessary duplication which could be expensive and time-consuming.

Proposed Course:

This project was discontinued upon reassignment of the Principal Investigator for duties at the Institute of Medical Microbiology and WHO Collaborating Center for Comparative Virology, University of Munich, West Germany.

Publications:

Ablashi, D.V., Aaslestad, H.G. and Easton, J.M.: Advances in research on oncogenic herpesviruses. In: Proc. Int. Workshop on Advanced Comparative Virology, UNI-Druck, Munich, West Germany, pp. 291-326, 1976.

Ablashi, D.V., Armstrong, G.R. and Easton, J.M.: Absence of horizontal transmission of Herpesvirus saimiri between experimentally infected and non-infected owl monkeys. Cancer Letters 2: 35-40, 1976.

Ablashi, D.V., Easton, J.M. and Guegan, J.H.: Herpesviruses and neoplasia in man and subhuman primates. Biomed. 24: 286-305, 1976.

Ablashi, D.V., Oie, H.K., Armstrong, G.R., Didier-Fichet, M.L., Tronick, S.R., Heine, U. and Dalton, A.J.: Xenotropic properties of an isolate from murine Rauscher leukemia virus in primates. J. Med. Primatol. 5: 223-236, 1976.

Armstrong, G.R., Orr, T., Ablashi, D.V., Pearson, G.R., Rabin, H., Loeb, W.F. and Valerio, M.G.: Characterization of Herpesvirus saimiri isolated from a chronically infected owl monkey. J. Natl. Cancer Inst. 56: 1069-1071, 1976.

Deinhardt, F. and Ablashi, D.V.: Epstein-Barr-like viruses of primates. W.H.O.-I.A.R.C.I.R.P./OHV Newsletter, No. 5, pp. 3-4, 1976.

Twardzik, D.R., Ablashi, D.V., Easton, J.M. and Armstrong, G.R.: Soluble complement-fixing antigens in Epstein-Barr virus cell system. Biblio. Haemat. 43: 412-416, 1976.

Sesterhenn, K., Krüger, G.R.F., Uhlmann, C., Ablashi, D.V., Samii, H., Wustrow, F. and Fischer, R.: Klassifikation maligner Lymphome des Halsbereiches: Kombinierte morphologische, immunzytologische, serologische und zellkultursuchungen. Laryng. Rhinol. 55: 823-832, 1976.

Ablashi, D.V., Armstrong, G.R., Fellows, C., Easton, J.M. and Twardzik, D.: Evaluation of the effects of phosphonoacetic acid (PAA) and 2-deoxy-d-glucose (2DG) on Herpesvirus saimiri (HSV) in Vero cells. In: Proc. of Third International Symp. on Detection and Prevention of Cancer, New York, New York, 1976, in press.

Ablashi, D.V., Easton, J.M. and Armstrong, G.R.: Oncogenic DNA viruses: experimental animals and in vitro systems for propagation, assay, transformation and tumor induction. In: Proc. International Symp. Experimental Animals and In Vitro Systems in Med. Microbiol., Munich, Germany, 1976, in press.

Ablashi, D.V., Twardzik, D.R., Easton, J.M., Armstrong, G.R., Luetzeler, J., Jasmin, C. and Cherman, J.C.: Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus-cell system. Europ. J. Cancer, 1977, in press.

Krüger, G.R.F., Samii, H., Sesterhenn, K., Uhlman, C.H., Ablashi, D.V., Fischer, R. and Wustrow, F.: Non-Hodgkin lymphomas: cell population and functional behavior. In Chierfelder, S., Rodt, H. and Thid, E. (Eds.): Immunol. Diagnosis of Leukemia and Lymphomas. J.F. Lehmanns, Verlag, Munich, Germany, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04876-05-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Oncogenic Virus Influence on the Biochemical Events of Host Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Ebert	Research Chemist	LDTV NCI
OTHER:	G. F. Vande Woude	Head, Virus Tumor Biochem. Section	LDTV NCI

COOPERATING UNITS (if any)

D. P. Tschudy	MET, NCI	R. Johnson	LEC, NCI
J. Lamon	MET, NCI	H. L. Bonkowsky	V. A. Hospital, White River Junction, VT
A. Desseroth	PO, NCI		

LAB/BRANCH: Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.3	1.1	1.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Infection of owl monkey kidney cells with Herpesvirus saimiri (HVS) induces the formation of a unique DNA polymerase with a molecular weight of about 190,000. The enzyme can be distinguished from the normal DNA polymerase by its template specificity for high G+C, its requirement for high salt and by its relative insensitivity to heat and high pH. The HVS-induced enzyme is resistant to the DNA polymerase inhibitor phosphonoacetic acid, while the DNA polymerase induced by herpes simplex is substantially inhibited by this drug. The G+C preferential template specificity of the HVS polymerase may suggest a mechanism for the generation and preferential selection of defective HVS DNA.

Friend leukemia cell (FLC) lines exhibited increased intracellular heme levels when incubated with DMSO or butyric acid alone, but no increase when these inducers were added together. DMSO increased the rate of butyrate uptake into soluble and acid-insoluble fractions of FLC. Procaine inhibited the stimulatory effect of butyrate. Porphyrin accumulation was considerably greater in DMSO-induced FLC than in butyrate-stimulated cells. These differential and opposing

effects of DMSO and butyrate on hemoglobin production suggest that these compounds have different modes of action.

Project Description

Objectives:

1. To examine enzymatic changes in host cells following infection with herpes viruses; to examine the properties of herpes virus-induced DNA polymerases and other enzymes responsible for viral DNA synthesis and to examine the activity of these enzymes on native and defective viral DNA templates.

2. To study the mechanisms controlling the differentiation of Friend leukemia cells as effected by drugs such as dimethylsulfoxide, butyric acid, and various agents which inhibit DNA synthesis. To investigate blocks or defects in the heme synthetic pathway occurring in drug-stimulated Friend leukemia cells.

Methods Employed:

Conventional biochemical procedures were utilized to follow alterations in heme synthesis occurring in cultured cells. Delta-aminolevulinic acid (ALA) synthetase activity was measured by an isotopic column chromatographic procedure. Hemoglobin was measured by a quantitative colorimetric method utilizing the benzidine reaction. DNA dependent-DNA polymerase was quantitated by measuring the incorporation of ^{3}H-deoxynucleotides into trichloroacetic acid-precipitable material. Herpesvirus saimiri-induced DNA polymmerase was separated from normal cellular DNA polymerase and DNase by column chromatography on DEAE-cellulose and phosphocellulose.

Major Findings:

1. Characteristics of the Herpesvirus saimiri-induced DNA polymerase: Infection of owl monkey kidney (OMK) cells with Herpesvirus saimiri (HVS) induces the formation of a nuclear virus-specific DNA dependent-DNA polymerase. The virus-induced DNA polymerase has a molecular weight of approximately 190,000 as determined by equilibrium sedimentation. The enzyme can be distinguished from the normal OMK DNA polymerase by its requirement for high salt and its relative insensitivity to either heat or high pH. The DNA polymerase shows a greater preference for "gapped" activated DNA than for poly(dC)·oligo(dG) than for poly(dA)·oligo(dT), suggesting that this virus-specific enzyme will more efficiently copy defective strands of herpes saimiri virus DNA which contain a high concentration of G+C. Neither normal OMK DNA polymerase nor the HVS induced DNA polymerase appear to be inhibited by phosphonoacetic acid (PAA), while the DNA polymerase induced by herpes simplex is substantially inhibited. However, HVS-infected OMK cells incubated in the presence of PAA continue to produce the virus-induced DNA polymerase even though the drug protects the cells from an HVS cytopathic effect suggesting that another viral function may be altered.

Friend murine leukemia cells (FLC) can be stimulated to produce hemoglobin in the presence of unrelated compounds such as dimethylsulfoxide (DMSO), butyric acid (BA), and inhibitors of DNA synthesis. The differential effects of DMSO and BA on hemoglobin production in FLC lines 745 and a mutant strain of 745, 585, was demonstrated. While both lines exhibited increased intracellular heme levels when incubated with DMSO or BA alone, no increase was observed when they were added together. DMSO increased the rate of uptake of BA into soluble and acid-insoluble fractions of FLC at 24 and 48 hrs. Maximum uptake of BA in both fractions occurred at 24 hrs and decreased by 48 hrs. Procaine inhibited the stimulatory effect of DMSO on heme synthesis in line 745, but increased the stimulatory effect of BA. Porphyrin accumulation occurring mostly in the form of protoporphyrin, was 6-10 times greater in DMSO-induced FLC than when induced in BA-treated cultures. Porphyrin accumulation was abolished in FLC dually treated with DMSO and BA. The opposing effects of these two inducers of hemoglobin synthesis in FLC suggest that these compounds have different site actions. No differences in oncogenicity between untreated and DMSO-treated FLC, T3-C12 cells was observed in DBA/2 mice. Both cells caused tumors and killed mice at about the same time. In contrast it has been reported that DMSO-treated, differentiated FLC are less malignant than untreated cells.

Significance to Biomedical Research and the Program of the Institute:

Study of herpes virus-induced enzymes can lead to a better understanding how DNA viruses replicate and how defective DNA is generated. Investigations on the mechanism of red cell differentiation in Friend virus-transformed cells can lead to a better understanding of viral transformation.

Proposed Course:

We will isolate highly-purified HVS-DNA polymerase. Since the HVS-induced DNA polymerase was not inhibited by phosphonoacetic acid, we will determine what steps or enzymes involved in HVS synthesis and replication are inhibited by this drug. Substrate specificity of the virus-induced DNA polymerase to DNA from various herpes viruses and their defective DNA will be examined. Antibody will be produced against purified HVS-induced DNA polymerase in rabbits.

Efforts will be directed towards elucidating mechanisms controlling erythroid differentiation in FLC. We will determine if a reverse transcriptase inhibitor occurring during differentiation of T3-C12 cells is also present in other lines and to clarify its role in differentiation. We will determine why inhibitors of DNA synthesis are effective in promoting hemoglobin synthesis in FLC.

Publications:

Ebert, P.S., Wars, I. and Buell, D.N.: Erythroid differentiation in cultures Friend leukemia cells treated with metabolic inhibitors. Cancer Res. 36: 1809-1813, 1976.

Bloomer, J.R., Bonkowsky, H.L., Ebert, P.S. and Mahoney, M.J.: Inheritance in protoporphyria. Comparison of heme synthetase activity in skin fibroblasts with clinical features. Lancet 31: 226-228, 1976.

Ebert, P.S. and Buell, D.N.: Viral reverse transcriptase suppression associated with erythroid differentiation of Friend leukemia cells. J. Natl. Cancer Inst. 58: 635-640, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04882-03-LDTV

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Events in Tumor Virus Replication and Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. F. Vande Woude	Head, Virus Tumor Biochem. Sect.	LDTV NCI
OTHER:	W. G. Robey	Research Chemist	LDTV NCI
	R. Ascione	Research Chemist	LDTV NCI
	P. S. Ebert	Research Chemist	LDTV NCI
	B. J. Graham	Staff Fellow	LDTV NCI
	Z. Bengali	Visiting Fellow	LDTV NCI
	P. J. Fischinger	Head, Tumor Virus Section	LVC NCI

COOPERATING UNITS (if any)

C. Long FCRC, Frederick, Maryland
B. J. Ortwerth University of Missouri Medical Center, Columbia, MO

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION

Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	0.5	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Moloney murine sarcoma virus specific P60 polyprotein contains p30, p15 and p12 polypeptides and is phosphorylated in transformed cells independent of virus assembly. Phosphorylation occurs in both threonine and serine residues of the p12 moiety of the polyprotein. P60 binds to single-stranded nucleic acids and can be isolated from cells by single-strand DNA sepharose chromatography.

Herpes simplex virus cellular RNA sequences can be enriched for and isolated from an excess of cellular RNA sequences by hybridization to denatured viral DNA immobilized on sepharose.

A DNA transfection assay has been developed using a combination of the calcium phosphate and DEAE dextran techniques and employs unique transfer of the transfected layer to susceptible indicator layer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To investigate biochemical events associated with tumor virus replication in relation to neoplastic transformation of host cells.

Methods Employed:

Oncogenic viruses are propagated in tissue culture. Focus-helper and plaque assays are used for the detection of some propagated and purified viruses. Other assays are: agarose gel diffusion for the detection of viral antigens; polyacrylamide gel electrophoresis and guanidine-agarose chromatography for the purification and identification of virus-specific proteins; in vitro labeling of purified viruses using radiolabeled amino acids and nucleic acids. Sensitive peptide mapping techniques are developed for use in comparing virion polypeptides. Endonuclease restriction enzyme cleavage of viral DNA and ethidium bromide agarose gel electrophoresis is used for mapping viral genes.

Major Findings:

The Moloney sarcoma virus (MSV) specific P60 was shown to be phosphorylated in transformed producer and non-producer cells demonstrating that RNA tumor virus polyproteins are phosphorylated as precursor molecules. The P60 as well as the p12 of MSV and of Moloney murine leukemia virus are phosphorylated in both serine and threonine amino acid residues and the absence of detectable phosphate in the p30 and p15 cleavage products of P60 suggest that the polyprotein is phosphorylated only in the p12 moiety. P60 was previously shown to bind to a wide variety of single-strand nucleic acids in a competition filter assay. Both virion and cellular P60 was retained on single-strand DNA sepharose columns and was eluted with 0.15 to 0.6 M KCl, demonstrating DNA binding by an additional criterion and showing that cellular P60 possess the same properties as the virion polyprotein. The basic protein, p10, of RNA tumor viruses is not phosphorylated, but does bind to single-strand nucleic acids with an affinity similar to the P60 binding. However neither p10 antigenic determinants nor tryptic peptides were detected in P60 which suggested that the binding was due to the unique configuration of the polyprotein or to a unique configuration of one of the constituent polypeptides, such as p12, in the polyprotein. The phosphorylation of P60 and its affinity for single-stranded DNA and RNA suggests a regulatory role for this protein. This function may be associated with a subunit of P60 but, as a stable uncleaved precursor in cells, its effect on cell metabolism may be associated with the transformation event.

A method was developed for isolating and purifying herpes simplex virus (HSV) specific RNA sequences from an excess mileu of cellular RNA sequences. Denatured HSV DNA (100 µg) coupled to CNBr sepharose (1 ml) was shown to specifically hybridize HSV RNA sequences even with a 10,000 fold excess of crude normal cellular RNA. All of the nonspecific RNA could be eliminated by repeated washings and the HSV specific RNA could be recovered intact by

elution with formamide. This method was used to isolate RNA from "early" and "late" HSV infected cells and should be useful for determining the genomic location in viral DNA of these RNA species. Using purified HSV DNA restriction fragments this method will be used to isolate the mRNA expressed in cells and ultimately used to recover sufficient mRNA to translate. This method was also used to examine several purportedly HSV-transformed cell lines and no RNA was detected above background levels.

The transfection of cells by herpes virus DNA has been more qualitative than quantitative and because of the cell-dependent, variable and deleterious effects of DEAE dextran and/or calcium phosphate on the transfected cell layer, provided less than ideal conditions for quantitation. Using isotopically labeled HSV DNA and by measuring biological activity, transfection assays were systematically and quantitatively analyzed for viral DNA, DNase sensitivity (an indicator of intracellular or extracellular transfecting DNA) and for plaque production. After examining many parameters the following conclusions and assay conditions were established. With the calcium precipitation method most of the transfecting DNA (70%) is attached to the cell surface by 72 hr and with either calcium or DEAE dextran methods, less than 3% of the transfecting DNA was intracellular (DNase resistant). The extracellular DNA is maximally active when 24 hr transfected cells are freshly plated onto a DEAE dextran-treated "indicator" cell layer. The primary transfected layer acts only as a vehicle for attaching DNA since heat-killed cells give approximately the same plaque titer as viable cells when plated on an "indicator" cell layer. By this technique, titers of HSV DNA from 600 to 1000 PFU/μg DNA can be routinely obtained on the same lot of DNA for periods greater than six months. This techinque provides a valuable tool for quantitating and monitoring viral DNA in transfection assays and was designed for the specific purpose of using one preparation of primary cells treated with restricted viral DNA to plate onto numerous secondary "indicator" cells to screen for cells with HSV transforming potential.

Significance to Biomedical Research and the Program of the Institute:

The tranformation of mammalian cells by sarcoma and DNA tumor viruses provides an excellent model for studying the molecular events associated with cell transformation. The unusual properties of MSV P60 may be related to the cell transformation event and as such will provide knowledge of how such transformations occur. The identification of a specific fragment of HSV DNA responsible for transformation makes possible the determination of the transformation antigens of HSV and provides a more specific probe for examining transformed human cells for evidence of HSV information.

Proposed Course:

1. The binding of P60 to nucleic acids will be further characterized and the exact interaction and its influence on DNA synthesis and transcription will be investigated.

2. The gene order for P60 will be determined and compared to MuLV precursors.

3. Fragments of HSV DNA will be utilized in transformation assays to identify fragments capable of transforming cells.

4. The gene products of this region will be identified from cell free synthesis of m-RNA specific for this fragment and by the PAGE immunoprecipitation technique using HSV specific antisera.

5. Evidence for the presence of this fragment or its gene products in human cancer will be investigated.

Publications:

Robey, W.G., Graham, B.J., Harris, C.L., Madden, M.J., Pearson, G.F. and Vande Woude, G.F.: Persistent herpes simplex virus infections established in two Burkitt lymphoma derived cell lines. J. Gen. Virol. 32:51-62, 1976.

Oskarsson, M.K., Scherer, M., Robey, W.G., Long, C. and Vande Woude, G.F.: Phosphorylation of Moloney murine sarcoma virus specific P60 and its DNA and RNA binding properties. In Clark, R.E. and Cumley, R.W. (Eds.): Yearbook of Cancer. Chicago, Yearbook Medical Publishers, 1976, pp. 504-506.

Vande Woude, G.F., Robey, W.G., Oskarsson, M.K., Haapala, D.K., Fischinger, P.J., Naso, R.B. and Arlinghaus, R.B.: Properties of Moloney sarcoma virus specific P60 and its detection in transformed cells. Bibl. Haemat. 43: 125-127, 1976.

Robey, W.G., Oskarsson, M.K., Vande Woude, G.F., Naso, R.B., Arlinghaus, R.B., Haapala, D.K., and Fischinger, P.J.: Cells transformed by certain strains of Moloney sarcoma virus contain murine P60. Cell 10: 79-89, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04884-04-LDTV

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Events of Herpesvirus saimiri Replication

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: J. A. Simonds Staff Fellow LDTV, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION

Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Herpesvirus saimiri infection of owl monkey cells results in a rapid inhibition of host DNA synthesis. Cellular protein synthesis is also inhibited, but less rapidly. Several new polypeptides can be identified in the infected cell cytoplasm that correspond in mobility to HVS capsid proteins.

The project was terminated prior to the present reporting period.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. Establish conditions for one step growth curve of Herpesvirus saimiri (HVS).

2. Characterize HVS-specific changes in DNA and protein during the course of infection.

3. Study the effect of DNA-binding antibiotics on the course of HVS infection.

4. Characterize DNA polymerase induced in owl monkey kidney (OMK) cells by HVS infection.

5. Characterize HVS DNA by cleavage with restriction enzymes.

Methods Employed:

OMK cells were infected with HVS at high multiplicity and pulse labeled with radioactive thymidine, uridine, and amino acids in order to identify viral specific macromolecules. Proteins were analyzed by electrophoresis in polyacrylamide slab gels and DNA analyzed in cesium chloride equilibrium gradients. Crude lysates from HVS infected cells as well as DNA polymerase activity purified by column chromatography on DEAE-cellulose and phosphocellulose were characterized as to optimal conditions of enzyme activity. Several different restriction enzymes were employed to characterize the susceptibility of HVS DNA to cleavage and to establish preliminary mapping of restriction fragments.

Major Findings:

Owl monkey kidney cells infected with high multiplicities of HVS (5:1) were examined for alterations in host cell DNA, RNA and protein synthesis. Host cell DNA synthesis was completely inhibited by 6 hrs p.i. as determined by blotting ^{3}H-thymidine-labeled Eco RI cleaved cell DNA. Typical HVS specific Eco RI DNA fragments could be observed by 12 hrs. Cellular protein synthesis was also inhibited, and ^{35}S-methionine-labeled infected cell cytoplasm examined by gel electrophoresis showed several new polypeptides approximately 130,000 daltons mol. wt. One of these polypeptides and several smaller infected cell polypeptides have the same mobility as polypeptides in purified HVS.

Sifnificance to Biomedical Research and the Program of the Institute:

Herpesvirus saimiri is oncogenic in certain subhuman primates and causes Teukemia and Tymphoma at 90% efficiency in certain species. As such it is a model system to demonstrate the oncogenic process in an animal whose physiology is very close to human.

Proposed Course:

Dr. Simonds left NIH prior to this reporting period. Therefore, this project was terminated on June 30, 1976.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04886-03-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetics of Herpesvirus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. J. Graham	Staff Fellow	LDTV NCI
OTHER:	G. F. Vande Woude	Head, Virus Tumor Biochem. Section	LDTV NCI
	Z. Bengali	Fogarty Fellow	LDTV NCI

COOPERATING UNITS (if any)

P. A. Schaffer Harvard University, Boston, MA

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.8	1.1	1.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The defective herpes simplex virus type 1 (HSV-1) DNA was physically mapped with restriction endonuclease (endo R) Bam HI and shown to consist of two unique fragments of identical molecular weight (1.2 megadaltons) and diffuse-microheterogenous fragments of 2 to 2.5 megadaltons. One of the small, unique fragments possessed the single endo R Eco RI site approximately 0.15 K base pairs from one end yielding a 1.1 megadalton fragment. Using "nick translated" defective DNA as a probe the origin of defective HSV-1 DNA was physically mapped in the right end 8 megadalton Hind III G fragment with endo R Bam HI.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To physically map herpes simplex virus (HSV) genome with restriction endonuclease (endo R) and to associate specific fragments with biological functions.

2. To determine the region of defective HSV DNA in the viral genome and to determine the mechanism by which defective genomes are generated.

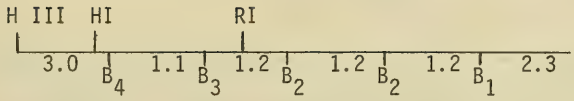
3. To determine whether normal HSV transformed cell DNA has nucleic acid sequence homology to specific regions of viral DNA.

Methods Employed:

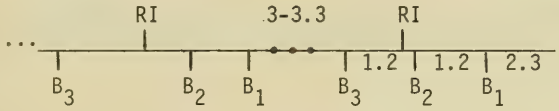
HSV type 1 defective DNA, generated by high multiplicities of infection, was characterized by cesium chloride isopycnic centrifugation and endonuclease restriction enzyme analyses. HSV DNA with high isotopic specific activity ($2\text{-}7 \times 10^7$ cpm/μg) was prepared by nicking with deoxyribonuclease and repairing with E. coli DNA polymerase using ^{32}P-labeled nucleotides. Endonuclease restriction enzyme Eco RI, Bam HI, Hpa I and Hind III were used to cleave viral and cellular DNA and the fragments were resolved on agarose and acrylamide gels. Nucleic acid homology was detected by filter hybridization using the Southern "blotting" technique.

Major Findings:

1. The right end of HSV-1 DNA was mapped with endo R Bam HI using defective DNA as a probe. By hybridization to filter paper transferred Hind III and Hind III-Eco RI endo R fragments of HSV-1 DNA, it was demonstrated that the Hind III G fragment (8 megadalton right end of HSV-1 half molar fragment) contained HSV-1 defective DNA sequences. The Eco RI K fragment (extreme right end 3.5 megadalton fragment) and the Hind III G - Eco RI K fragment (G-K, 4.5 megadaltons) were also positive by hybridization while the Hind III N fragment, the opposite end of the unique S region and adjacent to the repeated Eco RI K fragment was not. This result demonstrated that the defective HSV DNA originated from only one end of the unique S region since the G-K was hybridizable. To further characterize the origin of defective DNA the G-K and K fragments were isolated and additionally cleaved with Bam HI and Hpa I. Hybridization with defective probe to fragments obtained with Bam HI showed that fragments of K, 2-2.5 and 1.2 megadaltons were both positive and the 3.0 megadalton fragment was not. The K fragment was resistent to Hpa I cleavage while the G-K gave 2.0 and 3.2 megadalton fragments and only the 3.2 fragment was positive. In contrast all of the fragments generated by Bam HI and Hpa I from G-K and K were positive when total viral DNA was used as probe. These results suggest the following physical map for the Bam HI map of the Hind III G fragment of viral DNA:



Where H III is Hind III; HI is Hpa I; R is Eco RI and B is Bam HI. Similarly the physical map of defective DNA was mapped with Bam HI and Eco RI and the following map was derived:



The diffuse microheterogeneity obtained with Eco RI cleaved defective DNA (5.5-5.7 megadaltons) and that obtained with Bam HI (3.0-3.3 megadaltons) should represent the new junction established between the real right end of the HSV-1 DNA and the position in S where the defective terminates between the third and fourth Bam HI site. If either position is variable in the generation of defective DNA this could account for the microheterogeneity in genome size. The repeating unit in the defective DNA, of course, represents the distance from one junction to the next.

2. The DNA from normal mammalian cells was examined for the presence of related viral DNA sequences by DNA/DNA filter hybridization. The cellular DNA hybridized at least three percent of the input labeled viral DNA. The number of counts hybridized was consistently greater than twice background (determined with E. coli DNA). To determine whether specific host cell DNA fragments could be identified which hybridize with HSV DNA, cellular DNA was cleaved with various restriction enzymes, fractionated by electrophoresis in agarose, blotted and hybridized with isotopically labeled HSV DNA. Results to date have been variable, perhaps due to technical problems of the method. However because the sensitivity of the probe the positive experiments both by filter hybridization and by blotting would suggest that the hybridizable host DNA is a multiple copy species.

Significance to Biomedical Research and the Program of the Institute:

It has been shown that defective HSV DNA contains tandem repeats of 5% of the HSV genome originating from the terminal right end of the molecule. This DNA will be useful in studying the origin of HSV DNA replication and will give insight on how recombinational events occur between two DNA species. Since defective recombined viral genomes of certain viruses are responsible for transformation, this model system will be useful for showing how such molecules are derived.

Proposed Course:

The region of HSV-1 from which defective DNA originates has been mapped with restriction enzymes Bam HI and Hpa I. This information and the defective HSV-1 DNA will be used to determine whether sequences are homologous to HSV-2 and to map the homologous region in HSV-2. The origin of HSV DNA replication will be studied to determine whether defective DNA maps at this origin.

We will attempt to identify host cell DNA sequences homologous to viral DNA and to identify in viral DNA the actual sequences responsible for hybridization.

Publications:

Robey, W.G., Graham, B.J., Harris, C.L., Madden, M.J., Pearson, G.F. and Vande Woude, G.F.: Persistent herpes simplex virus infections established in two Burkitt lymphoma derived cell lines. J. Gen. Virol. 32: 51-62, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04895-05-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Characterization of Oncogenic Virus Polypeptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. G. Robey	Research Chemist	LDTV NCI
OTHER:	G. F. Vande Woude	Head, Virus Tumor Biochem. Section	LDTV NCI
	P. J. Fischinger	Head, Tumor Virus Section	LVC NCI

COOPERATING UNITS (if any)

F. C. Jensen	Scripps Clinic and Research Foundation, La Jolla, CA
C. W. Long	F.C.R.C., Frederick, MD

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	1.6	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A Moloney murine sarcoma virus (MSV) specific P60 polyprotein consisting of p30, p15 and p12 is detected in all cells transformed by the ml isolate of MSV. The observed properties of P60 in transformed cells may provide insight into the question of how virus transformation antigens are metabolized.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To develop biochemical and immunological methods to identify, isolate and characterize transformation specific viral polypeptides. Particular attention is given to the immunological detection and biochemical characterization of polypeptides associated with transformation. These methods will serve as tools for examining the molecular events associated with both viral replication and transformation.

Methods Employed:

Oncornaviruses were propagated in cell culture. Focus-forming assays, complement-mediated virus neturalization and lysis, electron microscopy, immunofluorescence and immunoprecipitation methods were used to determine the presence and extent of virus infection. Virion polypeptides were purified by guanidine-agarose chromatography and SDS-polyacrylamide gel electrophoresis. Specific antibodies to purified polypeptides were prepared in rabbits and goats. These antibodies were used to immunoprecipitate cell cytoplasms and fractions that bound to DNA-Sepharose columns. Immunoprecipitates were analyzed by electrophoresis and autoradiography. Polypeptide structure and antigenicity were analyzed by peptide mapping and gel diffusion methods. The virion location of structural polypeptides was determined by immune virolysis as measured by the release of virion RNA and RNA-dependent DNA polymerase.

Major Findings:

A Moloney sarcoma virus specific polyprotein with a molecular weight of 60,000 daltons, P60, was detected in the feline leukemia virus pseudotype of the m1 isolate of Moloney sarcoma virus, m1MSV(FeLV). The P60 was purified from virions and by peptide maps was shown to consist of the Moloney murine leukemia (MuLV) gag polypeptides p30 and p15. This was confirmed by gel immunodiffusion analyses of the multiple antigenic determinants present on P60 and indicated P60 was an uncleaved precursor to p30, p15 and p12. Specific antisera were prepared against P60 and used to immunoprecipitate cytoplasms prepared from cells transformed by MSV infection. In both producer and non-producer MSV transformed cells, P60 was the largest MSV polypeptide detected and was stable to cleavage in these cells. Intracellular p30 was not detected in cells producing heterologous virus indicating that P60 cleavage to p30 was associated with virus release. The p30 was detected in mouse cells producing MSV rescued with the homologous MuLV in addition to the expected precursor to p30, Pr70. In these cells the P60 was also detected as an uncleaved polyprotein. Five different mammalian species of m1MSV transformed cells were tested and P60 was detected in each. This indicated P60 was MSV-specific and the inability to detect p30 indicated that the molecular species responsible for murine group p30 antigenic reactivity of these cells was due to the presence of P60. To determine whether any of the gag gene polypeptides in P60 were located on the surface of RNA tumor viruses, the ability of anti-P60 serum to neutralize

and lyse virus in complement-mediated reactions was tested. In virus neutralization, the anti-P60 serum neutralized heterologous but not homologous RNA tumor viruses. Similarly anti-P60 in the presence of complement caused virolysis as measured by the release of virion RNA or by RNA-dependent DNA polymerase of heterologous RNA tumor viruses. The results indicated that interspecies antibody specificities directed against a gag product is located on the surface of RNA tumor viruses. Since anti-p30 serum had no effect, this suggested that either the p12 or p15 constituents of P60 may be responsible. To determine whether P60 was phosphorylated MSV transformed producer and non-producer cells were labeled with ^{32}P. In both cell types P60 was phosphorylated indicating that phosphorylation occurs independently of virus production. Since P60 was phosphorylated and was shown to bind to single-strand nucleic acids it may be involved in the regulation of cellular metabolism and contribute to viral transformation.

Significance to Biomedical Research and the Program of the Institute:

The detection of a stable uncleaved precursor to major oncornavirus structural polypeptides in MSV-transformed cells which bind to nucleic acids, provides a model system in which regulation of gene expression and viral transformation may be investigated.

Proposed Course:

Sensitive immunological and biochemical techniques will be used to study sarcoma virus defectiveness in order to obtain additional information about regulation of the sarcoma gene and the expression or maintenance of the transformed state.

Publications:

Robey, W.G., Graham, B.J., Harris, C.L., Madden, M.J., Pearson, G.F. and Vande Woude, G.F.: Persistent herpes simplex virus infections established in two Burkitt lymphoma derived cell lines. J. Gen. Virol. 32: 51-62, 1976.

Carlson, J.V., Robey, W.G. and Ortwerth, B.J.: Lysine tRNA's associated with avian myeloblastosis virus 70S RNA. J. Virol. 19: 760-763, 1976.

Oskarsson, M.K., Scherer, M., Robey, W.G., Long, C. and Vande Woude, G.F.: Phosphorylation of Moloney murine sarcoma virus specific P60 and its DNA and RNA binding properties. In Clark, R.E. and Cumley, R.W. (Eds.): Yearbook of Cancer. Chicago, Yearbook Medical Publishers, 1976, pp. 504-506.

Robey, W.G., Oskarsson, M.K., Vande Woude, G.F., Naso, R.B., Arlinghaus, R.B., Haapala, D.K. and Fischinger, P.J.: Cells transformed by certain strains of Moloney sarcoma virus contain murine P60. Cell 10: 79-89, 1977.

Vande Woude, G.F., Robey, W.G. Oskarsson, M.K., Haapala, D.K., Fischinger, P.J., Naso, R.B. and Arlinghaus, R.B.: Properties of Moloney sarcoma virus specific P60 and its detection in transformed cells. Bibl. Haemat. 43: 125-127, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1-CP-04897-05-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Interaction of Herpesvirus and Oncornavirus in Mixed In vitro Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. P. Andrese Staff Fellow LDTV, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Restriction analysis of plaque and host range variants of herpes simplex type 1 (mal strain) showed minor variations in fragment size in the region where the small unique and the terminal repeat are joined.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. Production and characterization of a HSV-1 syncytium-producing (syn) variant isolated from parental cloned, round-cell plaque-producing HSV-1 (syn^+) after several serial passages in JLSV9-RLV cells.

2. To determine the genetic relationship between syn^+ and syn.

Methods Employed:

Conventional tissue culture, viral, serological and electron microscopic procedures were employed. Extracted and purified viral DNA was examined by endonuclease restriction enzymes. Restriction enzyme-treated viral DNA was electrophoresed in 0.7% agarose for 20 hrs at 4 C and 80 v/cm.

Major Findings:

Several syncytium-producing (syn) variants of a cloned round cell plaque-forming HSV-1 (syn^+) (strain MAL) were obtained. Serial passage and plaque selection of syn^+ through mouse cells producing Rauscher leukemia virus yielded variant syn R; through Vero cells yielded syn V; and through a Burkitt lymphoma cell line yielded syn H. The variants and syn^+ were subsequently propagated in Vero cells, and the extracted viral DNA was examined by the endonuclease restriction (endo R) enzyme Eco RI. Of the fifteen DNA fragments generated, variations were detected only in fragments G and I. Because the syn locus has been characterized as a single point mutation and the G and I DNA fragments map adjacently in the HSV-1 DNA, the differences in G and I in the variants may indicate the position of the syn locus. Moreover, the differences between syn R, syn V and syn H in the size and amount of the G and I fragments suggests some host cell function in their formation.

Significance to Biomedical Research and the Program of the Institute:

The high frequency with which HSV-1occurs in the human population may allow this virus opportunity to superinfect cells already harboring another virus. As a result, intracellular interactions may result in genetic modifications expressed in its biological properties. Our studies also show that viral genetic alteration, indicated by the variation in the G and I fragments of three syn variants, may be host cell controlled. One of the objectives in the biological comparison of syn and syn^+ was to find natural, stable and reproducible markers or properties which could be used in studies of viral genetics. Since the syn locus has been reported to be a single point mutation and the G and I fragments appear to map close to one another in the syn^+ DNA, differences in G and I fragments of syn variants may provide a marker locating the syn locus. Thus far two markers have been identified: stability of the syncytium producing syn variant in three different cells and the ability of syn to replicate in CEF cells.

Proposed Course:

This project was terminated when Dr. Andrese left NCI on June 6, 1976.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04902-05-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

In vivo and In vitro Translation of Tumor Virus Specific Polypeptides

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Ascione	Research Chemist	LDTV, NCI
OTHER:	B. J. Graham	Staff Fellow	LDTV, NCI
	W. G. Robey	Research Chemist	LDTV, NCI
	G. F. Vande Woude	Head, Virus Tumor Biochem. Sect.	LDTV, NCI
	D. K. Haapala	Research Microbiologist	LVC, NCI

COOPERATING UNITS (if any)

B. J. Ortwerth University of Missouri Medical Center, Columbia, MO

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The site of synthesis and the location of the Moloney sarcoma virus (MSV) specific P60 polyprotein in subcellular fractions was determined in pulse-chase experiments. These studies showed that the P60 is synthesized in a low speed sedimenting membrane fraction and becomes associated with the nuclear fraction in a high salt releasable fashion. The latter is consistent with the P60 nucleic acid binding properties. The low speed sedimenting membrane polyribosomes, actively producing this polyprotein were isolated and the mRNA species purified by poly U-sepharose column chromatography. Cell-free translating systems using the homologous or heterologous wheat germ protein synthesizing factors were used to translate the P60 from subcellular fractions or mRNA from polyribosomes. The polyprotein was identified using anti-P60 serum and polyacrylamide gel analysis.

PHS-6040
(Rev. 10-76)

Position Description

Objectives:

To determine the molecular events associated with tumor virus genome expression by the specific translation of mRNA species associated with the transforming regions of viral genes.

Methods Employed:

In vivo pulse-chase analyses of Moloney sarcoma virus infected cells. The subfractionation of cellular components into nuclear and cytoplasmic polyribosomal components. The identification of virus-specific polypeptides using specific antisera and fractionation by polyacrylamide gel electrophoresis (PAGE). Subcellular fractions from infected cells active in the synthesis of virus specific polypeptides are isolated and used in cell-free translation systems and the related synthesized polypeptides are identified by immunoprecipitation and PAGE analysis. Infected cell polyribosomes are isolated and subfractionated by isopycnic and velocity-density gradients and the poly A rich messenger RNA isolated by poly-U-Sepharose chromatography. The mRNA from tumor virus infected cells is translated in cell-free protein synthesizing systems. The polypeptides synthesized are identified by immunoprecipitation and PAGE.

Major Findings:

1. In vivo pulse-chase experiments showed that the m1-Moloney sarcoma virus (MSV) specific polyprotein P60 was synthesized in a low speed membrane fraction. With detergent treatment this fraction yielded a large polyribosomal complex containing P60. During the chase portion of these experiments, P60 was shown to be abundant in the nuclear fraction and was not dissociated from the fraction by usual isotonic-detergent treatment, but was extracted only with high salt. In contrast the P60 was dissociated from the cyotplasmic fractions at low ionic strength. This suggests that the P60 is associated with nuclear chromatin and is consistent with the single-strand nucleic acid binding properties of the polyprotein. The association of P60 with the nuclear fraction was also resistant to RNase treatment suggesting either P60-protein or P60-DNA interaction. These data suggest that P60 may play some functional role in the nucleus which may be related to transformation by MSV. An MSV transformed cell low-speed membrane fraction was most active in cell-free protein synthesis using either a continuing dependent homologous or an initiation dependent heterologous translation system. More P60 immunoprecipitable material was synthesized in this fraction when compared with higher speed sedimenting subcellular fractions. These results demonstrated that the virus specific mRNA was predominantly associated with this fraction and that the association of P60 with the low speed membrane fraction did not occur as a consequence of the extraction method.

Poly-A rich mRNA was isolated from purified membrane-associated polyribosomes and utilized in cell-free translation. The P60 present in this product was detected by immunoprecipitation and PAGE. In addition, several higher

molecular weight immunoprecipitable polypeptides were detected which may be larger precursors that are rapidly degraded and therefore not discernable *in vivo*.

2. Unfractionated cytoplasmic RNA extracted from isotopically-labeled herpes simplex virus (HSV) infected cells was isolated by specific hybridization to HSV-DNA covalently attached to Sepharose. Control cell cytoplasmic RNA did not hybridize with the viral DNA and will provide a method for preparative isolation of HSV-specific mRNA for subsequent *in vitro* translation.

Significance to Biomedical Research and the Program of the Institute:

These studies should enable the identification of the polypeptides specified by RNA tumor viruses, and mRNA species which are post-transcriptionally processed *in vivo* to provide new initiation sites. This will be the only means for isolating these mRNA species since current technology does not allow *in vitro* mRNA processing. These same techniques will also be applied to the association of gene products with specific regions of the more complex herpes simplex virus.

Proposed Course:

The specific polypeptides associated with gene segments responsible for cellular transformation will be identified by using cell-free translating systems together with specific immunological reagents or with special techniques for isolating virus specific mRNA. Immunological methods will be used to specifically enrich virus specific polyribosomes and will serve as a source of mRNA for cell-free translation systems. These techniques are applicable to both RNA and DNA tumor virus systems.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04904-01-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cell Physiology on Oncogenesis: Mechanism of Transformation and Tumorigenesis by Tumor Viruses and Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Hatanaka	Head, Cell Physiology Section	LDTV NCI
OTHER:	B. Hampar	Head, Microbiology Section	LDTV NCI

COOPERATING UNITS (if any)
C. Boone LVC, NCI
T. Kakefuda CB, NCI

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Cell Physiology Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the project is to understand the mechanism of in vitro transformation and in vivo tumorigenesis of cells infected by tumor viruses and exposed to chemical and physical carcinogens. The research at present is to determine: 1) biological markers for cells during transformation and/or acquisition of tumorigenesis; 2) the role of the uptake of nutrients, particularly sugar transport during cell transformation and tumorigenesis; 3) expression of tumor virus genomes in cells; and 4) the mechanism of persistent infection of tumor viruses on the host cells.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The objectives are to study and understand the differences of physiology of normal and transformed cells so that basic information and findings obtained may be exploited to prevent and treat human cancer.

Methods Employed:

Standard methods of cell culture, virology, biochemistry, molecular biology and cell physiology were employed with modifications as required, with special emphasis on the use of radioisotopic tracers and molecular hybridization.

Major Findings:

1. Establishment of non-transformed cell lines. It is important to establish stable non-transformed cell lines as starting controls in order to study the cell physiology of the transformed state. It is well-known that most established cell lines available so far have a tendency to transform spontaneously after many passages and lose value as the control when compared to the cells transformed by carcinogens. We wanted to isolate cell lines that have stable properties with contact inhibition and anchorage dependency at the highest magnitude. The best way to get the cell lines, we thought, was to select surviving cells under the pressure of constant confluence, contrary to the selective method of 3T3 cells. We established the cell line, BLP, from BALB/c mouse embryo, and cloned sub-lines, particularly clones #3, 6, 7, and 8, to meet our criteria for non-transformed cells: contact inhibition, and anchorage dependency at the highest magnitude. These have been tested for tumorigenesis in vivo by conventional methods. After a three-month observation, all clones as well as the parental cell lines of BLP turned out to be non-tumorigenic in BALB/c mice. Therefore, we are convinced that the cells may be used to examine experimental tumorigenesis by viral, chemical and physical carcinogens.

We also found a non-transformed cells with a stable chromosomal number. An established line of muntjac cells from an Indian deer has seven chromosomes. A transformed muntjac cell clone, clone #10, with the same seven chromosomes, became tumorigenic as shown by tests in athymic nude mice. This may provide a model system for study of oncogenic mechanisms at the chromosomal level.

2. Establishment of transformed cell lines. For several years, Dr. Hampar Microbiology Section, has been investigating activation of xenotropic virus by certain DNA viruses that results in later cell transformation in vitro. He found that BLP cells as well as their clones are the ideal cells to observe the relationship between the activation of xenotropic virus and subsequent transformation following infection with DNA viruses. One of the cell lines, 16A, is a subline of BLP transformed by herpes simplex virus that is highly tumorigenic on BALB/c mice. By using this system, we are attempting to study sequential changes of cell physiology during transformation.

3. Cell physiology during and after transformation. We found that one of the remarkable changes of cell physiology during and after cell transformation is the uptake of nutrients. I reviewed these topics recently and chaired two sessions, namely, Oncogenic Transformation and Uptake Regulation, and Neoplastic Potential and Transport Regulation, in the International Symposium on Cellular Regulation of Transport and Uptake of Nutrients held at the National Institutes of Health, Bethesda, Maryland, February 26-27, 1976.

In the symposium I reported that uptake of sugars into cells by a saturable process increased enormously during and after transformation, and uptake by a nonsaturable process increased significantly but less remarkably compared to controls. The drastic change of uptake rates, observed at $5x10^{-3}$M sugar during and after transformation, emphasizes the significant observation that transition of the sugar uptake system from a saturable to a nonsaturable process occurs near the physiological concentration of D-glucose normally seen in animal blood. At concentrations higher than $5x10^{-3}$M, where a saturable process is barely involved, nonsaturable uptakes of D-glucose, D-mannose, D-galactose, 2-deoxy-D-glucose and 3-O-methyl-D-glucose proceed tens- to hundreds-fold faster than the rate of simple diffusion of L-glucose.

These findings suggest that nonsaturable uptake of the sugars known to be substrates for the saturable transport carrier system may not be a physical process or simple diffusion, as observed for L-glucose uptake. Rather, the nonsaturable uptake might be part of the total physiological process which, along with the saturable process, is controlled by a membrane-coordination mechanism.

A plausible mechanism was discussed in which negative cooperativity of nutrient uptake, such as that found in bacteria, is involved.

4. Expression and characterization of tumor virus genomes. We were interested in genetic expression of tumor viruses in the domestic cat. The domestic cat carries at least two families of type C RNA viruses: xenotropic RD114 virus and ecotropic FeLV (feline leukemia virus). We originally observed the presence of RD114 virus specific RNA in normal cat cells, although the function of the gene product remained to be examined. On the other hand, FeLV has been shown to be responsible for naturally occurring malignancies in domestic cats, and evidence for horizontal transmission has been presented. The question is raised whether uninfected cats have the FeLV related sequences in their cell DNA or not. A specific pathogen-free coloney of domestic cat was originated at the Ohio State University in 1966. Since its inception, there has been no evidence of FeLV or FeLV related disease in these animals nor have cats with neutralization antibody or antibody to the feline oncornavirus cell membrane antigen (FOCMA) been detected. Using tissues from this pathogen-free cat coloney, we found that the tissue DNA contained FeLV related sequences, demonstrating clearly that the origin of FeLV resided in the domestic cat DNA. As shown by domestic cats, type C viruses are associated in several species with naturally occurring malignancies and have been isolated from a variety of species,

including non-human primates. The ability to specify interviral relationships is crucial for evaluating the degree of relatedness of new isolates to known viruses.

Recently, Gallo's group reported the presence of a type C virus in cultured cells from a patient with acute myeologenous leukemia (AML virus). The AML virus (HL23V) was found to contain proteins of two primate virus groups, woolly monkey-gibbon virus and RD114-baboon virus by complement fixation and radioimmunoassay tests; the existence of two viruses was demonstrated by examining the host specificity of infection. We concluded that the HL23 virus preparation contained two distinct viruses: one indistinguishable from the woolly monkey virus, the second indistinguishable from an endogenous virus of the baboon species, Papio cynocephalus.

5. Mechanism of persistent infection of tumor viruses. Treatment with 5-BuDR of a non-producer BALB/c 3T3 clone transformed by Kirstein murine sarcoma virus yields various cell types with respect to their biological activity. Several distinctive cell lines, including persistently virus-producing lines, were cloned and characterized for their differential expression of viral DNA. Further analysis of viral sequences in the cellular DNA of these subclones by hybridization kinetics with complements of viral DNA, the copy numbers were significantly higher in persistently shedding lines than in non-virus producing lines. We concluded that the persistent production of RNA tumor viruses is due to the gene dosage effects of the proviral DNA.

Significance to Biomedical Research and the Program of the Institute:

Based upon this molecular characteristic of transformed cells, we may exploit enhanced sugar uptake as a method for detection of neoplastic cells. When 2-deoxy-D-glucose was used for the transport assay, the transported sugar was mostly converted to the phosphorylated product, 2-deoxy-D-glucose-6-phosphate, which accumulates inside the cells. Consequently, the difference in sugar transport between normal and tumor cells is enormously and irreversibly amplified. I proposed that this feature of the tumor cell membrane may be utilized for the detection of neoplasms in vivo. The accumulation of gamma ray emitting sugar such as ^{131}I-2-deoxy-D-glucose in tumor cells can be detected by gamma ray body scanning. Certain tissues with a high proliferation rate, i.e. gut epithelium, bone marrow and sexual organs, might produce high background levels. However, an unusual concentration of the isotope in certain localized areas can be readily recognized and further scrutinized as a suspected next of neoplasia. This has the virtue of being testable by radiologists who are immediately concerned with early detection of neoplasms in various species, including man, by the use of body-scanning techniques.

Proposed Course:

The search will be continued to determine biological markers correlated to the sequential process of transformation in vitro and tumorigenesis in vivo. Particular emphasis will be placed on the question of whether enhanced sugar

uptake observed during transformation in vitro may correlate to initiation of oncogenesis in vivo.

Publications:

Okabe, H., Twiddy, E., Gilden, R.V., Hatanaka, M., Hoover, E.A. and Olson, R.G.: FeLV-related sequences in DNA from a FeLV-free cat colony. Virol. 69: 798-801, 1976.

Okabe, H., Gilden, R.V., Hatanaka, M., Stephenson, J.R., Gallagher, R.E., Gallo, R.C., Tronick, S.R. and Aaronson, S.A.: Immunological and biochemical characterization of type C viruses isolated from cultured human AML cells. Nature 260: 264-266, 1976.

Hatanaka, M.: RNA dependent DNA synthesis. In Osawa, F. (Ed.): Genetic Information, Seibutsu Kagaku Koza III. Tokyo, Asakura-Shoten, 1976, pp. 86-114.

Hatanaka, M, Del Guidice, R. and Long, C.: A new quick screening of mycoplasma contamination in cell culture by adenine detection. Tissue Culture Association Manual 2: 437-438, 1976.

Hatanaka, M.: Saturable and non-saturable process of sugar uptake: Effect of oncogenic transformation in transport and uptake of nutrients. J. Cellular Physiology 89: 745-749, 1976.

Kakefuda, T., Lovinger, G.G., Gilden, R.V. and Hatanaka, M.: Electron microscopic studies of circular DNA in mouse embryo fibroblasts infected by Rauscher leukemia virus. J. Virol. 21: 792-795, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04905-01-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetics of Herpesvirus DNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Z. H. Bengali	Fogarty Fellow	LDTV, NCI
OTHER:	G. F. Vande Woude	Head, Virus Tumor Biochem. Sect.	LDTV, NCI
	B. J. Graham	Staff Fellow	LDTV, NCI

COOPERATING UNITS (if any)

D. Kingsbury University of California, Irvine, CA

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Herpes simplex virus (HSV) type 1 and 2 are serologically related but show major differences in the percent G+C, T_m and buoyant densitites. By hybridization HSV-1 and HSV-2 DNA are approximately 50% homologous. A sensitive hybridization procedure has been developed to map specific regions of homology and to physically relate the linear genomic maps of HSV-1 and HSV-2.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To standardize the method of hybridization of a nucleic acid probe to a fractionated pattern of restriction enzyme generated fragments of HSV DNA.

2. To study the regions of homology between HSV type 1 and 2 and to relate these regions to the linear genomic maps.

Methods Employed:

"Blotting" technique: Virion and cellular DNA are digested with restriction endonucleases and the resulting fragments are fractionated by agarose gel electrophoresis. The DNA fragments are denatured *in situ* and transferred to a cellulose nitrate membrane by elution. The transferred DNA fragments are then hybridized with a labeled nucleic acid probe and after removal of the unhybridized probe by washing and/or enzyme treatment the filter membrane is exposed to X-ray film. The DNA from HSV-1 or HSV-2 is cleaved with various restriction enzymes, used singly or in combination. The DNA generated fragments are fractionated by agarose gel electrophoresis and using the "blotting" procedure are hybridized with either the homologous or the heterologous "nicked translated" ^{32}P-labeled DNA.

Major Findings:

The "blotting" hybridization techinque has been developed as follows. The efficiency of transfer of DNA from agarose gels to filter membranes is better than 70% as monitored by the recovery of isotopically labeled fragment on filter paper. This filter can then be used in hybridization to determine the physical maps of viral genes, which viral genes are expressed during infection and/or transformation (coupled with the DNA sepharose hybridization procedure) or whether viral genes are present in transformed cells in an integrated state. Using nick labeled HSV-1 probe, all HSV-2 fragments generated by *Eco* RI, *Bam* HI, and *Hind* III hybridized to HSV-1 DNA indicating that the cross-type homology is uniformly distributed throughout the genome suggesting separate evolutionary pathways for these genomes with virtually no recent permissive recombinational occurrences. It has not been practical to compare homologous regions of HSV-1 and HSV-2 by the conventional "blotting" procedure since the complexity of endonuclease cleaved HSV DNA is too great and would require inordinate quantities of cleaved and isotopically labeled DNA. To circumvent this problem a double blotting technique has been developed utilizing conventional blotting in the first dimension. This blot pattern is then subjected to blotting under hybridization conditions to isotopically labeled HSV (either type 1 or 2) restricted DNA at right angles to the first blot. Homologous fragments appear as a "plus" on the autoradiograph greatly simplifying the comparison of genes. The efficiency of hybridization by this procedure is greater than by conventional blotting since the labeled DNA is in a high concentration during the hybridization blot.

Significance to Biomedical Research and the Program of the Institute:

Approximately 50% of the base sequences in the DNA of HSV-1 and HSV-2 are homologous. It has been shown that sequences common to both HSV types are transcribed in HSV transformed cell lines. This could mean that transforming genes by both types are homologous. Identification of the homologous regions will permit the testing of this hypothesis.

Proposed Course:

HSV-1/HSV-2 homology study: The regions of homology between HSV-1 and HSV-2 DNA will be precisely determined and HSV2 will be physically mapped.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04906-01-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
A. The Control of Expression of Early and Late SV40 Genes
B. Transcriptional Controls in SV40 and Hybrid Viral Genomes
C. Relationship of Human Papovaviruses to Human Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:		G. Khoury	Head, Virus Tumor Biol. Sect.	LDTV NCI
OTHER:	(A)	Y. Aloni	Visiting Scientist	LDTV NCI
	(A,B)	R. Dhar	Visiting Scientist	LDTV NCI
	(A,B)	C.-J. Lai	Expert	LDTV NCI
	(B)	F.-J. Ferdinand	Visiting Fellow	LDTV NCI
	(C)	D. Solomon		LDTV NCI

COOPERATING UNITS

(A)	E. May	Inst. der. Recherches, Scien. sur le Cancer, Villejuif, France			
(B)	E. Kuff	LB, NCI			
(B)	G. C. Fareed	U.C.L.A., Los Angeles, CA			
(C)	M. A. Martin	LBV, NIAID			
(C)	M. Israel	LBV, NIAID	(C)	S. Aaronson	LRTV, NCI
(C)	P. Howley	LP, NCI	(C)	K. K. Takemoto	LVD, NIAID

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
A-1.0 B-1.6 C-1.3	A-0.6 B-1.4 C-1.2	A-0.4 B-0.2 C-0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES (C) ☒ (c) NEITHER (A,B)

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. Temporal expression of SV40 genes occurs in two phases in the lytic cycle. During the early phase, prior to viral DNA replication, early SV40 RNA is synthesized which codes for the early viral T-antigen. Subsequently, late RNA, which codes for the viral structural proteins, is produced in large quantities (5 to 20 times as much as early RNA). The enzymic components responsible for the transition from the early phase to the late phase of the cycle are unclear, but were thought to be linked to viral DNA replication. We have shown, instead, that the abundant late transcription depends primarily on the presence of a functional T-antigen.

B. Using the sarkosyl extraction procedure with incorporation of radiolabeled nucleotides into nascent RNA in vitro, we have shown: 1) Virtually all of the RNA synthesized by the supernatant fraction is virus-specific; 2) qualitatively, the RNA synthesized by the sarkosyl pellet fraction is identical to the RNA synthesized by the supernatant; 3) the rate of synthesis of late:early RNA in an extract obtained late in the lytic cycle is approximately 10:1; 4) There is

essentially no initiation of transcription of viral sequences in vitro: 5) the method effectively eliminates processing of viral RNA, allowing for an analysis of nascent transcripts.

C. We have considered the possibility that human tumors may contain human papovavirus sequences which may or may not be etiologically related to the disease state. It has been shown, for example, that BKV and JCV, two human papovaviruses, are frequently found in immunodeficient patients, the latter case, in the brains of patients with PML. In addition, BKV has been isolated from a brain tumor of a patient with the Wiskott-Aldrich syndrome. We have been screening human tumors and continuous tissue culture lines derived from human tumors as well as normal human tissues for the presence of homologous sequences.

Project Description

Objectives:

A. To investigate the enzymatic functions responsible for temporal expression of the early and late SV40 genes.

B. To analyze the primary products of transcription of SV40 DNA as a means of determining (a) which transcripts are initiated on a temporal basis in lytically infected cells (b) which transcripts may be rapidly processed, and thus, not detectable in vivo.

C. An attempt to find DNA sequences homologous to human papovaviruses in normal or diseased human tissues.

Methods Employed:

A. Growth and plaquing of WT SV40, standard biochemical techniques for pulse-labeling and extraction of nucleic acid from infected cells. Nucleic acid hybridization assays including HAP chromatography, Southern transfer and hybridization to blots. Restriction enzyme purification and cleavage, densitometry, gel electrophoresis.

B. The extraction of viral transcriptional complexes using the sarkosyl or triton X-100 procedure. Purification of viral RNA as well as physical and hybridization analysis of these preparations by the usual biochemical procedures.

C. Nick translation procedure; blotting technique.

Major Findings:

A. Hybridization to the separated strands of SV40 DNA restriction enzyme fragments bound to nitrocellulose filters was employed to analyze the virus-specific cytoplasmic RNA synthesized in monkey cells after infection by wild type SV40 and the early SV40 temperature sensitive mutant tsA58. The fraction of SV40 specific RNA increased throughout infection with either

the wild type SV40 or with tsA58 in direct proportion to the accumulation of progeny DNA molecules, suggesting their role in the late transcriptional process. Cytoplasmic fractions from cells infected at various temperatures (31 to 41 C) by WT virus and harvested 48 hrs later contained 4 to 8% virus-specific RNA of which 5 to 10% was early SV40 RNA. In contrast, 5 to 8% of the cytoplasmic RNA from tsA58 infected cells incubated at 31.5 to 37 C for 48 hrs was virus-specific, but the fraction early virus-specific RNA ranged from 25 to 80%. In cultures incubated for 48 hrs at 41 C a temperature at which essentially no tsA58 DNA synthesis occurred, only 0.4% of the cytoplasmic RNA was virus-specific, but 90% of this was early RNA.

In other experiments cells were inoculated at 33 C and shifted at 48 hrs pi. to 40 C for various times. Of the virus-specific RNA, the percent early SV40 RNA ranged from 14 to 65% in tsA58 infected cultures. Analogous studies employing sarkosyl-extracted viral transcription complexes to incorporate label into nascent (unprocessed) viral RNA produced essentially identical results. This finding strongly suggests that the overproduction of early SV40 RNA occurs at the level of synthesis. While cytosine arabinoside effectively terminated most viral DNA replication in WT infected cells, the early:late RNA ratio remained less than 1:10. These results demonstrated that (i) the amount of virus-specific RNA synthesized depends directly on the amount of viral DNA available for the templates; once viral DNA replication has occurred the level of transcription remains high (ii) the termination of viral DNA replication does not terminate late SV40 transcription (iii) early SV40 RNA is overproduced by tsA58 at all temperatures, especially at higher temperatures. This overproduction of early SV40 RNA with increasing temperature appears to be correlated with a decrease in late viral RNA synthesis and an increase in the defectiveness of T-antigen. These results suggest that T-antigen regulates its own production either by repressing the synthesis of early viral RNA or by stimulating the synthesis of late SV40 RNA.

B. A nucleoprotein complex was isolated from SV40 African green monkey kidney (AGMK) cells by the sarkosyl extraction procedure (Gariglio and Mousset, 1975). The majority of the viral transcriptional activity appears in the sarkosyl supernatant where 70 to 90% of the labeled RNA is virus-specific. Although a low level of reinitiation of RNA synthesis was clearly demonstrated, none of this RNA was SV40-specific. The RNA synthesized by the purified transcriptional complex was analyzed by sucrose gradient sedimentation after denaturation and was found to be heterogeneous with a broad peak at 15 to 20S.

Hybridization experiments using the separated strands of SV40 DNA as well as restriction enzyme fragments have shown that *in vitro*-labeled RNA is synthesized from the entire late (L) and early (E) strand of the viral DNA. Between 80 and 85% of the virus-specific RNA was transcribed from the L-strand, a percentage similar but slightly lower than that found for the relative rate of transcription of the late and early viral DNA strands in lytically infected cells and in the nuclei of infected cells pulse-labeled *in vitro*. This suggests that *in vitro* RNA chain elongation with labeled

nucleotides reflects primarily the frequency at which transcription of the L- and E-strands is initiated in vivo. In addition, viral RNA synthesized from the chromatin fraction was qualitatively similar to that transcribed by the supernatant chromatin fraction. This observation would appear to exclude the possibility that a special class of viral RNA is transcribed from integrated viral templates.

In experiments with host-substituted variants of SV40 we could easily detect the transcription of the host sequences in vitro from a sarkosyl extract, whereas these RNA sequences were difficult to detect in vivo in nuclear pulse-labeled RNA. These data suggest that either host RNA sequences are rapidly degraded in vivo, or proteins are removed from transcriptional complexes by sarkosyl which permit "read-through" of an initiated viral RNA polymerase.

C. We have, using the nick-translation procedure, obtained viral DNA probes for SV40 and BKV with specific activities in the range of $5x10^7$ cpm/μg. These reassociate with roughly second order kinetics and can be used as rather sensitive probes for viral sequences in the human tumors with a sensitivity of less than one copy per cell. In addition we have been using the blotting technique to obtain similar information about the continuous human clones. To date, we have found no definitive results suggesting the presence of BK or SV40 copies in these tissues.

Significance to Biomedical Research and the Program of the Institute:

A. SV40 transformed cells in many respects represent a situation analogous to the early phase of the lytic cycle, with a block in viral DNA synthesis and the subsequent late events. Thus, it is of particular interest to determine the factors responsible for activating the expression of the late viral genes and provoking a full lytic cycle resulting in the death of permissive cells. We feel that the ability to make the transition to late phase of gene expression is the crux of what determines whether a viral genome will infect or transform a cell with which it interacts.

B. These studies are designed to investigate the role of transcription and posttranscriptional degradation of messenger RNA as a method of the regulation of gene expression, not only in SV40 infected cells, but in eukaryotic cells in general. We hope to gain an insight into how cells express normal gene function as well as how they might prevent expression of foreign DNA sequences.

C. A direct determination of the association, if not the etiological relationship, of human papovaviruses to human neoplastic disease.

Proposed Course:

A. We will pursue further studies using other temperature sensitive mutants of SV40 to demonstrate whether the synthesis of early viral mRNA is dependent on and regulated by the functional T-antigen. We will also attempt to determine if a similar type of regulation or viral RNA occurs in

SV40 transformed cells. Subsequently, we will attempt to show whether SV40 T-antigen interacts directly with viral DNA in a specific way, such as through initiation of RNA synthesis in regulating gene expression.

B. We will pursue these studies to determine the role of transcriptional versus post-transcriptional regulation of SV40 transcription. We will attempt to show that host sequences are either not transcribed in hybrid molecules or are rapidly degraded after synthesis. We will also use this approach in an attempt to define the early transcriptional intermediate and compare it to the late transcriptional complex.

C. We will continue this screening study. Assays will be made more sensitive by hybridization assays with restriction enzyme fragments of the papovavirus genomes. In addition, we will attempt to obtain a non-defective stock of JCV by DNA infection techniques.

Publications:

A. Martin M. and Khoury, G.: Integration of DNA tumor virus genomes. Current Topics in Microbiology 73: 35-65, 1976.

Avila, J., Saral, R., Martin, R. G. and Khoury, G.: The temperature-sensitive defects in SV40 group D mutants. Virology 73: 89-95, 1976.

Khoury, G. and May, E.: Regulation of early and late SV40 transcription: Dependence of late RNA synthesis on a functional T-antigen. J. Virol., 1977, in press.

B. Shih, T. and Khoury, G.: Isolation of viral specific RNA from SV40-infected cells by viral DNA chemically linked to a cellulose matrix. Biochemistry: 487-492, 1976.

Davoli, D., Ganem, D, Nussbaum, A. L., Fareed, G. C., Howley, P. M., Khoury, G. and Martin, M. A.: Genome structures of reiteration mutants of simian virus 40. Virology, 1977, in press.

Ferdinand, F.-J., Brown, M. and Khoury, G.: Synthesis and characterization of late lytic SV40 RNA from transcriptional complexes. Virology, 1977, in press.

C. Howley, P. M. Khoury, G. Takemoto, K. K. and Martin, M. A.: Polynucleotide sequences common to the genomes of simian virus 40 and the human papovaviruses JC and BK. Virology 73: 303-307, 1976.

Khoury, G. and Croce, C. M.: Assignment of the integrated SV40 DNA to human chromosome 7 in a SV40-transformed human cell line. Cytogenet. Cell Genet. 16: 164-170, 1976.

Khoury, G., Howley, P., Israel, M., Brown, D. and Lai, C.-J.: The papovaviruses: Their molecular biology and potential etiology in human disease. In Allison, F., Jr. and Rothschild, H. (Eds.): Human Diseases Caused by Cancer: Recent Developments, New Orleans, Oxford University Press, 1977, in press.

Khoury, G., Lai, C.-J., Brown, D., Israel, M. and Howley, P.: The human papovaviruses and their potential role in human disease. In Hiatt, H., Watson, J. D. and Winston, J. (Eds.): Origins of Human Cancer, Cold Spring Harbor, New York, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1-CP-04907-01-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

DNA Sequence of Control Regions of BKV

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Dhar	Visiting Scientist	LDTV NCI
	G. Khoury	Head, Virus Tumor Biology Section	LDTV NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	0.8	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of the work is to have a nucleotide sequence of some of the regulatory regions of BK virus genomes and compare these to the SV40 genome in order to better understand the gene structure and function of the papovaviruses. A detailed nucleotide sequence analysis has been performed on the segment of the BK virus genome that carries the origin of initiation of DNA replication. Sequences flanking both sides of the origins of replication of BK virus appear to have approximately 70 to 80% sequence homology with SV40 DNA.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To be able to demonstrate what type of sequence may be involved in the initiation of DNA replication; also, what sequence may be involved, if any, in determining the host range of these viruses.

Methods Employed:

Conventional nucleic acid sequence analysis of both DNA and RNA are being used. Hybridization of RNA to DNA on blots by published procedures is being used.

Major Findings:

BK virus, a human isolate, is a double-stranded DNA virus, approximately 5000 base pairs long. The initiation site for DNA replication has been localized within a restriction endonuclease fragment (Hind II-C) approximately 560 nucleotides long. We have undertaken a direct nucleotide sequence analysis of this fragment to compare with the DNA sequence of the initiation site for SV40 DNA replication. The sequence analysis suggests that 200 nucleotides of this Hind III endonuclease fragment of BK has approximately 70 to 80% sequence homology with a similar early region of SV40 DNA. No significant homology in this region was detected in previous hybridization experiments between BK and SV40 DNA when the results were analyzed by hydroxyapatite chromatography. The region of BK virus DNA comparable to the SV40 replication origin shows little sequence homology in a fragment of approximately 100 nucleotides but shows similar topological structure (like symmetrical regions). The sequence to the late region side of the SV40 virus DNA of approximately 200 nucleotides seems to have diverged considerably from the SV40 sequences, while in the latter part of the late region the BK Hind III-C fragment, there is marked sequence homology with the SV40 sequence.

Significance to Biomedical Research and the Program of the Institute:

It is remarkable that significant portions of the BKV DNA sequence are similar if not identical to SV40. And even where the sequences diverge near the replication origins, the general topological structure is the same. These sequence studies perhaps provide the best evidence for a close evolutionary relationship between BKV and SV40. We feel that a knowledge of primary sequence and DNA structure will provide an insight into the regulatory regions of DNA. We hope that a comparison of BKV and SV40 regulatory regions such as the initiation site for DNA replication might provide some information about the host range of these viruses, i.e. why BKV grows in human cells and SV40 in monkey cells.

Proposed Course:

This work was accomplished under a one year appointment for this Visiting Scientist. We propose to pursue the sequence studies of BKV in comparison to those of SV40 with the goal of defining important regulatory sites which may provide an insight into control or host range.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04908-01-LDTV

PERIOD COVERED
July 1, 1976, through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Post-transcriptional Regulation of SV40 Transcription

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Y. Aloni	Visiting Scientist	LDTV NCI
OTHER:	G. Khoury	Head, Virus Tumor Biology Section	LDTV NCI
	R. Dhar	Visiting Scientist	LDTV NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.1	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study is directed at an analysis of processing of the various SV40 specific mRNAs from their initial transcription until their translation on polysomes.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Specific objectives of this study are (a) to determine the number of SV40 primary transcriptional units in the nuclei of the infected cells and to localize their 5' ends on the physical map of the viral DNA (b) to quantitate the cytoplasmic viral mRNAs and to map their 5' end on the physical map of the viral DNA (c) to determine the pattern of processing of the nuclear viral RNAs (d) to determine whether RNase III type of enzyme is responsible for processing of the viral RNAs (e) to check whether drugs which interfere with the initiation of transcription would affect cellular mRNA transcription and not viral mRNA transcription and vice versa.

Major Findings:

We have previously shown that at least three populations of intranuclear virus-specific RNA exist late after infection (26S, 23S, 19S) and that these species are probably interrelated as precursors and products. We are presently involved in mapping these RNAs to show their specific relationship to each other, and the polarity of processing. In terms of positioning mRNAs and analyzing the process of their 5' termini, the demonstration of a 5' 7 methyl G, capping structure has provided an insight as well as a physical approach. We are presently involved in mapping the 5' capped end of the various SV40-specific RNAs. Since internal double-stranded RNA hairpin structures have been suggested as putative processing sites we are attempting to use RNase III to achieve specific processing of the larger SV40 RNA species. Finally we are studying the effects of the antimetabolite 5,6-dichloro-1-β-D-ribofluranosylbenzimidazole (DRB) to see if it has a differential effect on viral and host cell mRNA, or on specific viral mRNAs.

Significance to Biomedical Research and the Program of the Institute:

One of the central issues of viral carcinogenesis is concerned with the control mechanism of genetic expression in higher organisms. It is well accepted that neoplasia is associated with, or results from abnormalities of this control. The expression of viral genomes like SV40 in productively infected and transformed cells has several unique advantages as model systems for studying control mechanisms regulating eukaryotic gene expression: One advantage is the relatively high proportion of nuclear viral RNA transcripts which in the case of SV40 are processed to yield only three to five viral mRNAs. Another major advantage is the ease in obtaining quantities of viral DNA, which can be cleaved by restriction enzymes to a well characterized segments of the physical map of the DNA. Finally, various RNA-DNA hybridization techniques are now well-developed and highly sensitive, and they provide the technical tool which complement the above advantages. A determination of the role of post-transcriptional processing in the regulation of SV40 mRNA expression should provide an insight into how a viral genome might lead to an altered expression of the viral controlled cellular

genome, and in turn, how one might regulate the expression of genes in general, beyond the stage of primary transcription.

Proposed Course:

Appointment of this Visiting Scientist terminates in August, 1977.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1-CP-04909-01-LDTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Human Papovavirus BKV: Genetics, Biochemistry and Relationship to SV40

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.-J. Lai	Expert	LDTV NCI
OTHER:	G. Khoury	Head, Virus Tumor Biology Section	LDTV NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Virus Tumor Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	.9	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project involves studies of DNA tumor viruses of the papovavirus group and concerns mapping viral genes and regulatory signals for the normal viral development and oncogenic function in mammalian cells. Experiments include transformation of human cells by a human virus, BK virus and mapping of the transforming virus to determine the nature of mutation in the BK viral genome for effecting a stable association with, and a subsequent malignant change of its natural host cell. The respective functions for oncogenesis and viral DNA replication of BK virus and SV40 will be compared with regard to their sequence relatedness and complementation properties. Studies also center on mapping and sequence analysis of control signals of the viral genes in order to understand their regulatory mechanisms for gene expression.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1) To map genomic region of BK viral functions responsible for (a) viral DNA synthesis during lytic production, and (b) establishment of a stable virus-cell association and subsequent neoplastic change of human cultured cells.

2) To study the interrelationship of BK virus and SV40 with respect to the extent of nucleic acid sequence homology, functional complementation properties, and oncogenesis in mammalian cells.

3) To elucidate viral regulatory signals for gene expression with emphasis on the production and maturation processes of various classes of SV40 mRNA by mapping responsible genomic regions and nucleic acid sequences.

Methods Employed:

Transformation of human cells by U.V. mutagenized BK virus; assay for the presence of BK genome in transformed human cells; rescue of transforming virus by induction techniques; mapping of mutation sites in the viral genome; isolation of BK defective and conditional lethal mutants; complementation analysis between BK virus and SV40; construction of SV40 deletion mutants by excision with restriction endonucleases; analysis of SV40 mRNA by mercury-agarose gel electrophoresis.

Major Findings:

1. Studies on mapping the structural genes of SV40 using temperature-sensitive and deletion mutants have revealed the genomic organization and gene expression of the virus. These studies showed that T-antigen, found in SV40 induced tumor and in lytically infected cells, is coded by approximately one-half of the genome corresponding to the early region as defined by transcriptional mapping. The other half of the genome, the late region, codes for viral structural proteins. The genomic map of SV40 appears to represent the general gene organization for other members of the papovavirus group, including polyoma virus of mouse, and BK and JC virus of humans. Further studies by nucleic acid hybridization between SV40 and BK virus showed that in addition to 20% strong homology there is a total of more than 50% weak homology in both early and late regions, suggesting that these two closely-related viruses might have similar biological functions, and might share certain functional properties.

2) We have considered the possibility that transformation of human cells by human virus BK might occur if BK virus was mutagenized to inactivate any cytolytic function of the virus. Transformation assays of human embryonic kidney cells infected with U.V.-irradiated BK virus have been carried out and several putative transformed human cells were isolated. These transformants are being further characterized for the presence of the BKV genome.

Several experiments are presently under way to study the biology of the "BK-virus transformed" cells and the transforming virus.

3. To examine regulatory mechanisms of viral gene expression in mammalian cells, deletion mutants of SV40 lacking sequences in suspected control regions have been constructed and cloned. These mutants have been analyzed for the production of viral mRNA. The results from mercury-agarose gel electrophoresis of RNAs showed that a class of deletion mutants lacking a portion of a structural gene produced altered mRNAs of a molecular weight corresponding to the length of the remaining portion, suggesting that the deleted genome can be transcribed and the transcript processed normally. However, another class of deletion mutants produced no detectable abnormal cytoplasmic RNA, indicating an alteration in the transcription or perhaps post-transcriptional processing might have occurred. More mutants of both classes are being constructed to define the limits of DNA segments which either lead to the production of altered transcripts or to the absence of a stable RNA.

Significance to Biomedical Research and the Program of the Institute:

Studies on genetics of SV40 have revealed that the general genomic organization and gene expression are essentially similar among members of DNA tumor viruses of the papovavirus group. Preliminary results have indicated some close relationships between simian virus 40 and human BK virus. Further investigations on the aspect of BK virus-human cell stable association, such as transformation, should help to demonstrate (a) the potential oncogenicity of BK virus in vitro, (b) mechanisms of induction responsible for possible transmission; and, (c) physiological interactions which occur between the resident virus and the cell. The goal of these studies is to understand the control mechanisms involving viral gene expression in mammalian cells, especially as this project relates to a human virus which is potentially oncogenic.

Proposed Course:

As has been demonstrated by others, BK virus appears to complement the A gene defect of SV40 in monkey kidney cells, although more rigorous tests using BK mutants remain to be carried out. Several studies are in progress to determine the level at which this complementation takes place. Human cells transformed by BK virus will be further characterized. Further studies on regulatory mechanisms of virus gene expression in mammalian cells will be conducted.

Publications:

Khoury, G., Lai, C.-J., Brown, D., Israel, M. and Howley, P.: The human papovavirus and their potential role in human disease. In Hiatt, H., Watson, J. D. and Winston, J. (Eds.): Origins of Human Cancer, Cold Spring Harbor, New York, 1977, in press.

Khoury, G., Howley, P., Israel, M., Brown, D. and Lai, C.-J.: The papovaviruses: Their molecular biology and potential etiology in human disease. In Allison, F., Jr. and Rothschild, H. (Eds.): Human Diseases Caused by Cancer: Recent Developments, New Orleans, Oxford University Press, 1977, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04929-09-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Herpesviruses: Immunologic and Biologic Studies in Relation to Human Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. Hampar	Assistant Chief	LDTV, NCI
		Head, Microbiology Section	LDTV, NCI
OTHER:	A. Brown	Sr. Dental Surgeon	LDTV, NCI
	J. R. Stephenson	Acting Head, Viral Genetics Sect.	LRTV, NCI

COOPERATING UNITS (if any)

J. G. Derge	FCRC, Frederick, Maryland
A. Boyd	FCRC, Frederick, Maryland
M. R. Tsai	FCRC, Frederick, Maryland

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Microbiology Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, FCRC, Frederick, MD 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purpose of the project is to study the relationship between herpesvirus and human cancer. The major topic of interest concerns the mechanism of in vitro cell transformation by herpes simplex virus (HSV) and the putative co-carcinogenic role of HSV reflected in its ability to alter expression of endogenous type C virus information.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To study the mechanism of cell transformation by herpes simplex virus (HSV), including the ability of HSV to alter the expression of endogenous type C virus information.

Methods Employed:

Standard tissue culture, virologic, and biochemical methods were employed with modifications as required.

Major Findings:

1. Cell transformation by herpes simplex virus (HSV). Studies from several laboratories have clearly shown that infection of certain cells (particularly those of rodent origin) with UV-irradiated HSV (UV-HSV) may result in cell transformation with at least some cells acquiring malignant properties. Two theories have been proposed to explain the putative oncogenic potential of HSV. The most widely held view places HSV in the same category as papovaviruses which are considered direct oncogens in that specific transforming gene sequences present in the virion are integrated into the cell genome and are responsible for transformation. The second viewpoint, and the one we espouse, holds that HSV functions as a co-carcinogen by altering expression of endogenous type C virus information. Under these conditions, persistence in transformed cells of HSV genetic sequences may not be required and transformation would occur by a hit-and-run mechanism.

Previous studies from this laboratory have established that infection of BALB/c mouse cells with UV-HSV and synthesis of the type C virus persists for only 1-2 days. Extensive tests have established that the type C virus activated by UV-HSV in BALB/c cells is xenotropic in host range.

The initial studies which demonstrated the ability of UV-HSV to activate type C virus employed a non-producer line of BALB/c cells (K-BALB) transformed by the Kirsten murine sarcoma virus (Ki-MSV). Activation of type C virus with concomitant rescue of the Ki-MSV genome was detected by infectious center formation using permissive rat cells. These studies have now been extended to sarcoma-negative BALB/c cells as a preliminary to correlating type C virus activation with transformation by UV-HSV. Two cell lines have been employed. The first (11c) is a clonal isolate of BALB/c embryo cells which display transformed properties. The 11c cells grow in agarose and are contact inhibited. Tumors develop in x-irradiated syngenetic BALB/c mice, but not in unirradiated animals. The 11c cells have proven particularly useful in the development of techniques for studying type C virus activation by UV-HSV in cells lacking a rescuable sarcoma virus, although they are not useful for transformation studies. An infectious center assay was developed for studying type C virus activation in UV-HSV infected 11c cells based on findings originally reported by Fischinger et al. that non-producer feline

(F81) cells form foci when infected with mouse xenotropic but no ecotropic type C virus. When 11c cells infected 20-24 hrs previously with UV-HSV are treated with mitomycin and seeded on polybrene treated F81 cells, discrete foci develop in 8 days which can be quantitated for determining the frequency of type C virus activation. The infectious center assay has proven highly reproducible and works equally well with HSV type 1 or 2. The findings to date are in agreement with those reported previously with UV-HSV infected K-BALB cells in that type C activation occurs at a maximum frequency of 1-1-3x10^{-4}, depending on the UV dose and multiplicity of infection.

The second cell line (BLP) was isolated by Dr. M. Hatanaka (NCI) from BALB/c embryo cells. This line was chosen from many tested for use in transformation studies. The properties of the BLP cells include: a) contact inhibition, b) inability to grow in agarose or agar, c) lack of tumorigenicity when inoculated in irradiated or unirradiated BALB/c mice, and d) absence of detectable spontaneous type C virus activation.

Activation of type C virus (xenotropic) can be readily demonstrated in UV-HSV infected BLP cells by an indirect technique whereby mitomycin treated cells are co-cultivated with feline F81 cells and the medium is tested after 2-3 weeks for sarcoma virus. Development of a direct infectious center assay with BLP cells is presently being pursued.

Since the main objective of our program is to determine the mechanisms of cell transformation by UV-HSV, with particular emphasis on a putative co-carcinogen role, extensive preliminary studies were carried out to obtain a line of BALB/c cells which would give reproducible results in transformation assays. Of numerous cell lines tested, the BLP cells were chosen for additional study. The results of experiments to date with BLP cells and UV-HSV may be summarized as follows. First, morphologic transformants appear within 6-10 weeks after infection with UV-HSV and following 2-3 cell passages. The transformed colonies show variability with respect to their degree of loss of contact inhibition and cell morphology, suggesting that transformation by UV-HSV is a multi-step process. Parallel studies employing chemical carcinogens show similar variations in properties of transformed cells. Second, transformation of BLP cells by UV-HSV or chemical carcinogens can also be detected by growth of colonies in agarose. This criterion seems more sensitive and less time consuming than awaiting the appearance of transformed foci. Third, transformation by both UV-HSV and chemical carcinogens is characterized by cells acquiring the ability to grow first in agarose and then in agar. With continued passages of the total cell population or isolated foci, the cells acquire the ability to grow in agar. Tumorigenicity studies in irradiated and non-irradiated BALB/c mice are presently underway. Finally, transformation by UV-HSV appears correlated with type C virus activation in that cultures which ultimately show transformants after 6-10 weeks also show type C virus activation on day 1 after infection with UV-HSV. Although a direct cause and effect relationship has yet to be established, the results to date are consistent with the suggestion that HSV may serve as a co-carcinogen by altering expression of type C virus information.

2. Detection of type C virus activation by UV-HSV. Procedures employed, thus far, for the detection of type C virus activation by UV-HSV required the synthesis of complete infectious virions detectable either directly in infectious center assays or indirectly following rescue and pseudotype formation with sarcoma virus in permissive cells. The possibility was considered that type C virus activation by UV-HSV may occur in many more cells than being detected by these procedures. Preliminary studies indicated that little, if any, type C virus was being released into the medium of activated cultures, which precluded detection by reverse transcriptase assays. Studies were carried out utilizing immunofluorescent (FA) and radioimmunoassay (RIA) procedures for detection of type C viral p30 antigen.

When 11c cells were infected with UV-HSV and tested by infectious center formation and FA the following results were obtained. The optimal time of UV-irradiation for maximum levels of type C virus activation was determined for each multiplicity of infection employed. With HSV type 1, for example, maximum activation occurred with 6 min irradiated virus. Staining by FA with anti-HSV sera was observed in approximately 13% of cells infected with 4 min irradiated HSV and this percentage declined until by 10 min irradiation no staining was detectable. Parallel cells when stained with rabbit anti-MuLV p30 serum or normal rabbit serum showed staining in 2% of the cells infected with 4 min irradiated virus, and this declined to undetectable levels with 8 min irradiated virus infected cells. The fact that staining by the anti-MuLV p30 was not correlated with activation measured by infectious center assay and staining was also observed with normal rabbit serum suggested that the FA staining with both of these sera was nonspecific. This was tested further using mouse cells (BALB/c and NIH Swiss) productively infected with live HSV. The results indicated significant nonspecific staining of HSV infected mouse cells by various rabbit sera. Non-specific staining was most prevalent with HSV type 1 infected cells, while HSV type 2 infected cells showed low but significant levels of non-specific staining. The non-specific staining was not removed by absorption with HSV or RLV infected cells, although specific antibodies to HSV and RLV were effectively removed by the absorption procedures. In contrast to the results with mouse cells, non-specific staining of HSV infected Vero cells was observed at low to negligible levels with various rabbit sera.

Additional studies were carried out to confirm the results by FA. Mouse cells (BALB/c and NIH Swiss) productively infected with HSV type 1 or 2 were harvested at 8 and 24 hrs and tested by RIA for p30 protein. If the intense FA staining we observed in productively infected cells by the anti-MuLV p30 sera was due to p30 antigen, we should have seen a significant rise in p30 protein levels by RIA. In contrast, we observed no significant difference in p30 levels in 8 hr infected cells and actually observed a 5-fold decrease in p30 levels in 24 hr infected cells. These results were consistent with the known ability of HSV to shut down cell metabolic processes during productive infection.

A final experiment was carried out with BALB/c cells infected with UV-HSV and harvested at 24 hrs for testing by RIA. The conditions employed resulted in type C virus activation detectable by infectious center formation. The results by RIA, however, indicated a slight decline in p30 levels compared to control uninfected cells.

Two important conclusions can be drawn from these studies. First, in contrast to reports from other laboratories, we found no evidence for the induced synthesis of type C viral p30 antigen in mouse cells productively infected by HSV. The FA techniques employed by others gave erroneous results due to the non-specific staining incurred in HSV infected mouse cells with rabbit sera. Second, type C virus activation in UV-HSV infected BALB/c cells does not occur in significantly more cells than we detect by infectious center formation.

3. Biochemical transformation by UV-HSV. Utilizing a thymidine kinase (dTK) deficient line of NIH Swiss cells (N C1 A cl 10) obtained from Dr. R. Goldberg (NCI) we have isolated a number of transformed clones utilizing selective HAT medium. Procedures have been developed for identifying HSV-specific dTK by gel electrophoresis and the transformed clones are presently being tested to verify the transformed state. These biochemical transformants will be compared to UV-HSV induced malignant transformants with respect to the state of the HSV genome.

4. Detection of DNA binding proteins in HSV infected cells. Preliminary studies have been completed for the identification and eventual isolation of DNA binding proteins in HSV infected cells. The role of these proteins during productive infection will be elucidated as will their possible significance in transformation induced by UV-HSV.

Significance to Biomedical Research and the Program of the Institute:

Elucidating the mechanism of cell transformation by HSV may reveal a situation whereby an infectious, normally cytolytic DNA virus affects expression of an endogenous, vertically-transmitted RNA virus which acts as a direct oncogen. If a co-carcinogen role for HSV proves valid, it would raise the possibility of transformation by a hit-and-run mechanism whereby persistence in the transformed cells of HSV gene sequences would not be required.

Proposed Course:

Studies will be continued to elucidate the mechanism of transformation by HSV. Particular emphasis will be placed on the question of whether HSV functions as a co-carcinogen by altering expression of endogenous type C virus information.

Publications:

Hampar, B., Aaronson, S.A., Derge, J.G., Chakrabarty, M., Showalter, S.D. and Dubb, C.Y.: Activation of an endogenous mouse type C virus by ultraviolet-irradiated herpes simplex virus type 1 and 2. Proc. Natl. Acad. Sci. U.S.A. 73: 646-650, 1976.

Rand, K.H., Reed, B., Broseus, I., Hampar, B., Gilden, R. and Long, C.: Mixed-culture cytopathogenicity between KC and XC oncornavirus indicator lines and "virus-free" human choriocarcinoma cells. J. Natl. Cancer Inst. 56: 509-512, 1976.

Hampar, B., Hatanaka, M., Aulakh, G., Derge, J.G., Lee, L. and Showalter, S.D.: Type C virus activation in "non-transformed" mouse cells with UV-irradiated herpes simplex virus. Virol. 76: 876-881, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04943-03-LDTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A Possible Co-carcinogenic Role of Herpesvirus and Chemical Carcinogens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A. M. Brown Dental Surgeon LDTV, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of DNA Tumor Viruses

SECTION
Microbiology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, FCRC, Frederick, Maryland 21701

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project was discontinued upon relocation of the Principal Investigator at the beginning of the present reporting period

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04943-03-LDTV

Project Description

Objectives:

1. Elucidation of the possible role of herpes simplex virus antigen as a promoting agent for chemical carcinogens.

2. Elucidation of the role of soluble herpes simplex virus antigen adsorbed on the cell surface in the elicitation of a T-cell reaction as measured by tumor graft rejection and microcytotoxicity assays *in vitro* using cells transformed by chemical carcinogens *in vivo* and *in vitro*.

Methods:

Standard immunological procedures.

Major Findings:

This project was discontinued upon relocation of the Principal Investigator at the beginning of the present reporting period.

Significance to Biomedical Research and the Program of the Institute:

These studies had been initiated to help determine the nature of the association of herpes simplex virus with cellular transformation.

Proposed Course:

This project has been terminated.

Publications:

None

SUMMARY REPORT

b. LABORATORY OF RNA TUMOR VIRUSES

July 1, 1976 to September 30, 1977

Office of the Chief

Beginning early in 1975 the Laboratory of RNA Tumor Viruses (LRTV) scientists made cause and prevention of cancer in experimental animals their No. 1 objective. In 1976 and 1977 sufficient information on the role of virogenes and oncogenes as possible causes of spontaneous and chemically-induced cancers provided sound bases for exploring the feasibility of immunoprevention of cancer with the use of antiviral and anti-oncogene specific immunization. This was made possible by recent decisive data provided by basic biological and molecular studies on the part of many investigators in the Viral Oncology and Virus Cancer Programs which demonstrated the oncogenic roles of endogenous genetically transmitted virogenes, especially the oncogenic recombinant viruses in viral permissive systems (W. Rowe and J. Hartley, NIAID), and the more elusive role of the wholly endogenous oncogenes which are now known when expressed to specify the transforming proteins that serve in non-viral permissive systems as endogenous causes of cancer in mice, rats, and probably most other animals. Thus the existence of endogenous oncogenic genes (termed sarcogenes, leukogenes and carcinogenes) have been established and characterized in experimental animals to an extent that it is now not only possible to use this information to prevent cancerous changes in non-viral permissive oncogene systems such as we report in the rat (vide infra), but to anticipate similar experimental studies in higher primates and in man. In fact, the rat model which we describe in the text below provides an excellent model for protection in non-viral permissive systems such as we clearly have in the human species, wherein overt expressions of endogenous viruses have not been demonstrated despite enormously extensive investigation.

Investigations in LRTV during the last two years suggest that prevention of spontaneous and chemically-induced cancer might turn out to be a relatively straightforward and rather simple accomplishment, provided one had in hand immunogenic preparations specific for tumor cell antigens held in common by the various neoplasms of a given species.

In the following report I will describe several different approaches to immunoprevention of cancer in mice and rats that have proven to be successful. The success that has been achieved has in large part been due to extensive collaboration between LRTV scientists, many colleagues in other Laboratories in the Viral Oncology Program, Litton Bionetics, Frederick Cancer Research Center, associates at the University of Southern California, Scripps Institute, and The Jackson Laboratory. The immunoprevention experiments described below were done in collaboration with Drs. R. Gilden, D. Fish, D. Djurickovic and R. Toni, Frederick Cancer Research Center; Dr. Jack Gruber, NCI;

J. Lemp, Electro-Nucleonics; R. Trimmer and P. Hill, LRTV; and B. Sass and C.Whitmire, Microbiological Associates.

Experiments begun in FY 1975 to prevent cancer in experimental animals (chiefly mice and rats) reached maturity in FY 1976 and FY 1977, thus we are now able to report significant immunoprevention (1) of spontaneous leukemias in AKR mice and in AKR crossbreds, (2) of chemically-induced sarcomas in C3H/f and C57Bl/J mice, and (3) of a variety of ordinarily lethal transplantable tumors and cancer cells of the rat.

Prevention of Leukemias with Passive Immunity

Immunoprevention against leukemias which reach incidences of 90 to 100 percent in AKR mice at a year of age was achieved with the use of virus specific immunoglobulin (IgG) prepared in goats against the AKR and other ecotropic viruses; three replicate experiments each gave significant protection. Instead of 90 to 100 percent of the normal AKR mice dying of leukemia by 365 days, 80 percent or more of the mice immunized with high titers of passive antibody (>1:1600) at birth to 14 days of age not only survived 365 days, but many survived as long as 450 days.

Protection Against Leukemias with Live Vaccine

The use of combined killed and live vaccines given at critical time periods in several AKR crossbreds (AKR X C57L, NIH and SWR) begun in 1975 have thus far provided significant protection ($P < 0.003$) against leukemia at 450 to 500 days of age in the C57XAKR F_1 progeny; similar protection appears to be likely in the NIH Swiss mice, but not in the SWR crossbreds. Failure in the latter can be explained by unique viral and genetic factors in the SWR mice which specify a variety of cancers distinct from that specified by the AKR and C57L mouse systems.

Prevention of Chemically-Induced Sarcomas in C57Bl/6 and C3H/f Mice

Several years ago in several collaborative experiments with Dr. C. Whitmire of Microbiological Associates, we reported that both killed and attenuated live C57Bl/J virus (radiation leukemia virus of Lieberman and Kaplan) given prior to 3-methylcholanthrene injections successfully prevented 60 to 80 percent of the expected subcutaneous sarcomas.

The rationale behind the immunization of C57Bl/6 mice with killed and live vaccines came from early observations that C57Bl/6 mice developed exceptional endogenous immune responses to various types of stimulus including (1) radiation (observed initially by H. Kaplan), (2) 3-methylcholanthrene (observed by H. Igel and R. Huebner), and (3) the prophylactic inoculation of C57Bl/6 mice with low dosages of endogenous RadLV (observed by C. Whitmire and R. Huebner).

Whitmire and Huebner published several papers in 1971 and 1972 reporting in each case significant protection against cancer in C57Bl/6 with the use of a formalin-killed vaccine and subsequently a low dosage live virus vaccine. Similar but antigenically distinct immunizing viral preparations did not protect against 3MC tumors in this system.

Protection Against Chemically-Induced Cancer (3MC) using the same Passive IgG Immunization which Prevented Spontaneous Leukemia in AKR Mice.

Recently we were successful in achieving protection against 3MC-induced cancers in several experiments in C3H/f mice using the same passive antibody (IgG); the protection against chemically-induced sarcoma was equivalent to that achieved with the same IgG in the AKR virus permissive system.

These findings are exciting for several reasons: first of all because the same passive immunity (IgG) was found able to prevent both spontaneous leukemia and chemically-induced sarcomas. Although an unexpected event, further evaluation tests revealed that protection was provided only when the anti-RadLV IgG had neutralizing antibody titers which were equally very high to both ecotropic and xenotropic viruses (1:1600 to 1:3200). This observation suggested that the RadLV virus may be very much like the recently discovered Rowe-Hartley AKR recombinant oncogenic virus. We have since confirmed this assumption in that we have recently acquired a virus non-producer RadLV lymphoma carrying antigenic expressions held in common with non-producer mouse sarcomas.

This is still hypothetical and much investigation will be required to elucidate the precise protective moieties; however, it seems likely that the RadLV IgG antibodies include in addition to virogene the antibodies to the RadLV leukogene and/or sarcogene. Other possible hypotheses do not satisfactorily explain the clearcut and repeated protection observed. It should be mentioned that potent passive IgG's versus the xenotropic C57L/J virus and to Rauscher leukemia virus which has high level antibodies to both ecotropic and xenotropic viruses also protect against the chemically-induced cancers in C3H/f mice.

There are a number of interesting questions raised by these various successful methods for preventing cancer in mice:

1. What is being suppressed by the active and passive antibodies to endogenous murine viruses? Our observations suggest that the immune responses are suppressing or modifying Akv-1 and Akv-2 virogenes of the mouse (Rowe and associates).

2. What is it that suppresses the development of leukemias and sarcomas? Only one explanation comes to mind: profound suppression of virogenes by IgG early in the life of AKR mice may prevent the development of sufficient virus to form the ecotropic-xenotropic recombinant which Rowe and Hartley have documented to be necessary for the creation of the "oncogenic" AKR virus.

As mentioned above, the mechanism by which passive antibodies to virogenes can suppress chemically-induced tumors in virus free C3H/f mice is unknown, but it seems likely that the IgG prepared against RadLV virogenes also includes antibodies to expressions of the murine oncogene probably also embodied in the RadLV (vide supra).

Although much remains to be elucidated concerning mechanisms involved in immunoprevention of cancer in mice, it is hard to ignore repeatable experimental tumor protection systems since they offer unparalleled opportunities for elucidating mechanisms.

Immunoprevention of Experimental Cancer in Rats

Rats in general and Fischer inbred rats in particular show little or no spontaneous expressions of type C RNA tumor viruses (RaLV) during their natural 4-6 year life span; however, they do develop lymphomas and other cancers spontaneously with advancing age and respond regularly to chemical carcinogens that lead to cancers which are also nearly always devoid of any detectable RaLV. Thus, in these respects the readily overt tumors of Fischer rats appear to behave like those in humans in that human cancers develop or are induced without any attendant expressions of overt virus before, during or after tumor development. However, the discovery of a tumor antigen held in common by all rat tumors provides unique and exciting possibilities for transplantation tumor immunity.

In order to develop a suitable approach to preventing cancer in rats, we have taken advantage of several additional opportunitues: (1) The isolation of Kirsten sarcoma virus (Klement, Kirsten, Huebner), and Scolnick's demonstration that this KiMSV virus contains a DNA sarcogene. (2) The isolation by Rhim of a non-producer, KiMSV(NP), cell which carries antigens specified by the rat sarcogene and which cross reacts with common rat tumor antigens. (3) Our finding that the KiMSV(NP) tumor cell produces tumor antigens held in common by not only other KiMSV(NP) cells but also to other rat tumors as follows: (a) spontaneous lymphoma, (b) rat cells spontaneously transformed in culture (Price and Freeman), (c) chemically-induced tumors and transformed cells (induced by 3MC, 4NQO, DMH), and (d) DNA virus-induced tumors and transformed cells (polyoma, adenoviruses, SV40, bovine papilloma).

The rat tumor antigen which is identified in CF and RIA and held in common by all known rat tumors is in itself a very unique observation, since numerous reports (Foley, Prehn, Klein and others) have emphasized that every tumor (including multiple tumors in the same animal) have individual humoral and transplantation antigenic determinants. This observation continues to be true as far as it goes. However, during our extensive transplantations of various rat tumor cells, including those transformed spontaneously and those transformed by polyoma, 4NQO, and RNA sarcoma viruses, each were found to have a common tumor antigen when tested by antisera from rats carrying these various tumors. The common tumor antigen does not cross react and presumably is not related to the rat type C virogene, but is fully reactive with the antigens of KiMSV(NP) sarcogene transformed cells.

Protection against transplantation of otherwise lethal doses of rat tumor cells is accomplished by preimmunizing the rats with 10^6 to 10^7 KiMSV(NP) transplantable tumor cells. Although the KiMSV(NP) cells replicate as a subcutaneous tumor, the tumor is completely rejected within 2-3 weeks, following which a powerful cell-mediated immunity prevents the establishment of other ordinarily highly lethal rat tumor cell transplants given at cell dosages of 10^6; thus quantitatively significant protection can be reproducibly demonstrated. Although the 10^6 cell challenge may break through occasionally, the protection observed is highly significant ($P < 0.001$). Since the KiMSV(NP) induced cell-mediated immunity against lethal rat tumor cell challenge is not only profound and long lasting (up to 70 days or more), we are now testing the ability of the KiMSV(NP) sarcogene immunity to prevent induction of cancer in rats by 3MC, DMH, and radiation. Of special interest was the observation that tumors induced by different types of tumors--a 4NQO-induced sarcoma, colon cancer, a carcinoma induced by DMH, and a spontaneous lymphoma in an old rat--were all prevented from transplanting in the KiMSV(NP) immunized rats, while lethal in control rats. These unique observations suggest that effective immunity to oncogene sequences and proteins may be sufficient to prevent all histotypes of cancer as long as the cell-mediated immunity persists. We are now exploring methods for inducing persistent long-acting cell-mediated immunity to rat tumors.

We believe these findings suggest an important conclusion as follows: within the cells of a given host system (mouse, rat and perhaps humans) there exists an all purpose oncogene capable of producing sarcomas in mesenchymal cells, lymphoma in lymphoblasts and carcinoma in epithelioid cells. The corollary of this furnishes the concept that for prevention of any type of cancer in rats we may be aiming at a target which consists of expressions of a single endogenous oncogenic genome. If confirmed, this information would simplify enormously conceptual and operational approaches to identifying both the cause and prevention of cancer. Thus, cancer may not represent 100 different diseases as some oncologists have claimed. Also the concept of "thousands of causes of cancer" could be modified with the knowledge that in a given species a successful response to the expressions of perhaps a single oncogene might prevent cancers representing a variety of different histotypes.

Prospects for future studies are focused on studies of the causes of cancer in higher primates and hopefully also in man. Our initial plans are to produce predictable numbers of cancers in higher primates (baboons and chimpanzees) with the purpose of identifying common tumor antigens and specified by endogenous oncogenic sequences and resulting common tumor antigens. Research approaches to control of human cancer would also be focused on the identification of common tumor antigens in human cancers which would then be studied by heterotransplantation of the human cancers in immunosuppressed rats and/or hamsters. Extensive absorption of suitable reactive antisera acquired from tumor bearing rats and hamsters conceivably could identify a common tumor specific antigen in human tumors. Such antigen(s) combined with inducers of cell-mediated immunity could provide opportunities for immunization of humans having the highest risks for developing cancer.

Molecular Biology Section and Viral Genetics Section - (Heads, Drs. Aaronson and Stephenson)

Type C RNA viruses were shown to exist as endogenous viruses within the cellular genomes of many mammalian species. A major research effort is aimed at devising immunological approaches for detection of endogenous virus expression in human tissues. Techniques were successfully developed for the detection and partial purification of several type C virus-coded proteins from virus-negative cells of a variety of species. During the past year, efforts have focused on the search for viral antigens in primate tissues. It has been possible to show that species representing each of the major genera of Old World primates express antigens related to the major structural proteins of the baboon-RD114 virus group. With highly sensitive immunological techniques now available, analogous approaches are being utilized in the search for evidence of endogenous virus expression in higher apes and man.

A viral etiology to cancer would be strongly implied by prevention of tumors through immunological approaches directed against virus infection and/or endogenous virus expression. Recent studies, performed in collaboration with Drs. Kelloff and Stephenson, have led to the demonstration that the incidence of radiation-induced leukemia in the C57/816 strain can be significantly reduced by passive or active immunization against the mouse type C virus. These findings help to demonstrate that the endogenous viruses are etiologically involved in cancers.

A new group of vertebrates has been shown to contain endogenous viruses. It has been possible to induce type C virus from cells of a New World mammal, Columbian black-tailed deer (O. hemionus). Viral cDNA prepared from this endogenous virus has been shown to detect related genetic sequences in a wide variety of ungulates, including those of both New and Old World origin. The evidence indicates that endogenous viruses have been genetically transmitted in these mammals for several million years and have existed in the New World for a period of at least five to ten million years.

The development of immunologic approaches for study of different proteins of mammalian type C viruses has led to the establishment of immunoassays for each of the known viral-coded proteins. These included p30, p15, p12, and p10, each of which is coded as part of a polyprotein precursor of the gene, designated gag. The viral reverse transcriptase (pol gene) and the envelope glycoprotein, gp70 (env gene) have all been characterized physically and by radioimmunoassay. The virus-coded nature of each protein as well as their unique antigenic determinants have been established. In the broadest interspecies immunoassays for each of these viral proteins, it has been possible to demonstrate that interspecies antigenic determinants are shared between the analogous p30s, p10s, p15s, and reverse transcriptases, respectively, of all known mammalian type C viruses. These findings strongly argue for a common progenitor in the evolution of mammalian type C viruses.

In studies by others, cats and gibbon apes have been shown to contain high-titered antibodies to horizontally transmitted oncogenic type C viruses. To date, human sera have not shown evidence of exposure to these viruses by

analogous immunological assays. Included have been sera from over 200 laboratory workers involved in viral oncology research. The fact that reactivity to type C viral proteins has not been detected in sera of normal or cancer patients tested argues that classical horizontal infection with known mammalian type C viruses must be rare.

The relationship of FOCMA to known FeLV structural proteins has not been previously clarified. In studies performed in collaboration with Dr. Essex, it was possible to demonstrate that the antibody to FOCMA was independent of antibody to either of the major FeLV structural proteins, p30 or gp70. These findings argue strongly against the possibility that FOCMA is either of these FeLV-coded structural proteins.

In studies designed to elucidate the structure of the type C viral genome, the order of the gag gene of mammalian type C viruses has been established. The presence of specific virus-coded proteins in different MW precursors in cells transformed by replication-defective transforming viruses and cells infected with a leukemia virus mutant, temperature-sensitive for cleavage of the gag gene precursor, has been analyzed. These findings made it possible to deduce the order of the murine viral gag gene as 5'-p15-p12-p30-p10-3'.

A radioimmunoassay has been developed for antibody directed against the major 24,000 molecular weight (p24) protein of bovine leukemia virus (BLV).

Analysis of aroung 6000 sera from randomly selected dairy cattle from 21 States of America indicated the presence of antibody against BLV p24 in a high fraction of animals tested. The percentage of animals with detectable antibody to BLV ranged from a low of 2-4% to as high as 42%. Moreover, sera from a significant number of antibody-negative animals were shown by competition immunoassay to contain high levels of viral antigen. These findings indicate that the infection by this virus may be much more prevalent among domestic cattle than previously believed.

Human sera from 450 individuals employed in slaughterhouses or on dairy farms as well as over 900 sera from patients with various forms of cancer failed to demonstrate detectable antibody to BLV p24.

Viral Immunotherapy Section - (Head, Dr. Pearson)

During the year, a new spontaneous leukemia (L76) and a mammary carcinoma in strain 2 guinea pigs were discovered and are currently serving as model systems in support of the ongoing common antigen and chemo-immunostimulation investigations. Preliminary histopathological studies revealed that the L76 leukemia exhibited a striking hemotological and pathological similarity to acute monocytic leukemia occurring in man.

(In collaboration with B. Barlow, S. Aaronson, S. Tronick, K. Perk.) During the previous year, a cloned guinea pig cell line was isolated which produced substantial quantities of GPV without any requirement for induction with halogenated pyrimidines. When these cloned cells were exposed to graded concentrations of either dexamethasone or halogenated pyrimidines, no

significant increase in virus production, compared to the controls, was observed. This indicated that, in contrast to MMTV production in mouse cells, the glucocorticoid hormones do not affect GPV production. Furthermore, it appears that the level of virus production is at essential maximal levels. This was verified by isolated 54 subclones of this cell line following three cycles of extensive UV irradiation of monolayers of the virus producing clone (designated 118/11). Of the 46 subclones tested for reverse transcription activity, only 4 had enzyme levels higher than the parent cell line. This might well be due to an increase in gene dosage, rather than an alteration of the control mechanisms affecting virus production.

Viral Immunology Section - (Head, Dr. Kelloff)

In the past year, several major studies that are part of an integrated vaccine program have been completed. These include: a study showing reduction of X-ray induced leukemias in C57BL mice by induction of active immunity or transfer of passive immunity to endogenous type C virus. Active immunity was established by immunization with inactivated R-MuLV and passive immunity by transfer of goat anti-G-MuLV immune IgG. Both regimens resulted in measurable circulation antibody to AKR-gp70 throughout part of the latent period of tumor development and both resulted in reduced tumor incidence compared to several controls.

Virus and Disease Modification Section - (Head, Dr. Chirigos)

Pyran copolymer was found to be equal to or better than Freund's complete adjuvant when used as an adjuvant with irradiated tumor cell vaccines, thus it appears to be one of the most potent agents capable of exerting antitumor and immune modulating activity. Results of a study conducted with C^{14} labeled pyran copolymer show pyran is concentrated mainly in the liver and spleen, tissues which are heavily populated with reticuloendothelial and lymphocytic cellular elements. Employing biochemical procedures, pyran copolymer was found to derepress cellular DNA synthesis which may account for the larger number of macrophages and other lymphoid cellular elements observed in pyran treated animals.

Animal Virology and Field Studies Section - (Head, Dr. Sarma)

B-lymphocyte cultures were established from spleens of BALB/c, C57Bl/6, NIH Swiss, and SWR mice of various age groups. Spontaneous, consistent and thus predictable release of a B tropic mouse endogenous virus occurred from the very first passage in cultured lymphocytes derived from BALB/c mice six months or older but not from similar lymphocytes derived from BALB/c mice of 1.5 months or 3 months of age. C57Bl/6, NIH Swiss and SWR mice belonging to various age groups ranging between 1.5 months and 18 months failed to exhibit such a spontaneous release of viral particles. We conclude that in BALB/c splenic B-lymphcytes, a breakdown of cellular control mechanisms occurs in older animals leading to viral production while such a phenomenon is absent in C57Bl/6, NIH Swiss and SWR mice.

REV was produced in quantities suitable for isolation and purification of the major group specific (gs) antigen. This antigen was purified by column chromatographic techniques from disrupted virus. Polyacrylamide gel electrophoretic analysis revealed that the preparation was greater than 95% pure. Using this antigen, we developed a radioimmunoassay using the rabbit antiserum against the purified gs antigen. Currently experiments are in progress to test the cross-reactivity of this antigen with other known avian viral gs antigens. Also, the assay is being used to test the presence of antibodies against this gs antigen in sera of turkeys which are (a) normal, (b) experimentally infected with REV, and (c) naturally infected with REV.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04831-06-LRTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Reticulum Cell Neoplasms Type B in BALB/c Mice.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. Ruth Merwin, Research Biologist, LRTV, NCI

OTHER: Dr. R. Asofsky, Chief, LMI, NIAID

Dr. P. Campbell, Chemist, LMI, NIAID

COOPERATING UNITS (if any)

K. Sanford, LB, NCI

LAB/BRANCH Office of Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Virus and Disease Modification Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
National Cancer Institute, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In the course of studies on various aspects of reticulum cell neoplasms type B that arise in old BALB/c mice many tumors have been transplanted and are being maintained in mouse passage. B lymphocytes were suspected of being the cell of origin of these tumors; therefore, in a collaborative study the transplanted tumors were tested with immunofluorescence techniques for the presence on cell surfaces of immunoglobulins characteristic of B lymphocytes. Also the cells were tested to determine whether the Ig was secreted into the surrounding medium. Correlations were sought between the type of Ig on the surface of the tumor cells and the site of tumor growth in either the mouse in which the tumor arose or in mice that received transplants of the tumors.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To continue studies on type B reticulum cell neoplasms that arise spontaneously in old BALB/c mice, conducting tests to characterize the reticulum cell tumors with regard to their histology, preferential site of tumor growth and further characterize the tumor cells.

Methods Employed:

Immunofluorescence methods were employed. See methods reported in Project No. Z01-A1-00134-15 for details and methods of testing for secretion of immunoglobulins by cells.

Major Findings:

1. Thirty-six tumors were tested for the presence of cells with Ig of the G, A or M types. They were also tested with a polyvalent reagent which reveals the presence of Ig of a yet unidentified type or types. For 24 of these tumors 65 to 100 percent of the cells were positive for G, A, M or the polyvalent reagent. Four were positive for G, one for G and M, five for A, eleven for M, and three for the polyvalent test only. For twelve tumors the percent of cells that were positive ranged from 0 to 60 and most of the Ig positive cells were M or an unidentified type.

2. The cells of these tumors do not secrete Ig.

3. The amount of local growth either at the subcutaneous site of inoculation of tumor cells or on the peritoneal surfaces after intraperitoneal inoculation as compared to the growth in the spleen, lymph nodes or liver varied markedly with the percent of B cells present in the tumors. Growth was almost completely local for five tumors among nine that had only 0 to 30 percent B cells whereas none of the tumors with 65 to 100 percent B cells showed such localized growth. There was also a difference, although less marked, between tumors of the G and M types. All four of the G tumors showed considerable local growth as well as some growth in spleen or nodes or liver. Only one among eight of the M tumors showed as much local growth.

4. The sites of tumor growth in the mice in which tumors arose showed a correlation with the type of Ig on the cell surface in one situation. Of the eight tumors characterized by predominantly enlarged lymph nodes in mice where tumors originated, four were among the five IgG tumors and four were among the 29 other tumors.

Proposed Course:

1. To determine whether the immunoglobulins are synthesized by the tumor cells or are passively acquired.

2. To determine why some of these tumors have so few B cells; whether they have a T cell origin or are B tumors that are particularly antigenic or have large numbers of macrophages present.

3. When it has been demonstrated which of these tumors are composed of cells that are synthesizing the immunoglobulins found on the tumor cell surfaces these tumors should be characterized in many ways including their homing pattern, their response to X-irradiation and to a variety of chemotherapeutic agents.

Significance:

Preliminary observations indicate that the A and M immunoglobulins are synthesized by the tumor cells. If so these tumors are of value to serve as models for the expression of Ig on B lymphocyte cell surfaces. These tumors may also serve as models for experimental purposes of human B cell tumors of the A and M types.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04875-14-LRTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Control of Oncogenic Viruses and Induced Diseases Mediated by External Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. M. A. Chirigos, Head, Virus and Disease Modification Section, LRTV, NCI

OTHER: Dr. W. A. Stylos, Expert, LRTV, NCI

Mr. R. M. Schultz, Technician, LRTV, NCI

COOPERATING UNITS (if any) Dr. K. Paucker, Med. Col. PA, Phil. PA.; Dr. T. Theodore, LM, NIAID; Dr. P. Chretien, DCT, NCI; Dr. R. Adamson, LCHPH, NCI; Dr. J. Luetzler LVC, NCI; Dr. U. Heine, LVC, NCI; Dr. T. Chang, M, LNC; Col. Blasco, Armed Forces Rad. Res. Inst.; Dr. P. Price, Micro. Asso., Inc.

LAB/BRANCH Office of Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Virus and Disease Modification Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
National Cancer Institute, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Eight agents have been demonstrated to possess immunoadjuvant activity (5 chemicals and 3 biologicals). In vitro studies with pyran copolymer, glucan, and purified interferon show that they directly activate naive macrophages to exert a tumoricidal effect. A marked correlation has been demonstrated for the capacity of pyran copolymer and glucan to enhance antitumor resistance in the host and their ability to activate macrophages in vitro. Histopathological studies show that pyran treatment not only increases macrophage function, but also mobilizes macrophages to migrate to the primary and disseminated tumor sites exerting tumoricidal effects. Activation of macrophages by polyanion adjuvants may be mediated through an interferon mechanism rather than a cellular metabolic product or as yet unidentified material. Polyanions are interferon inducers. Interferon per se activates macrophages indicating that interferon may be acting as a lymphokine.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The overall goal of this project is to determine the most effective means of preventing and/or controlling cancer. Viral induced, carcinogen induced, and spontaneous occurring tumors serve as animal model systems to assess the most feasible approach that can be employed to exert maximal antitumor therapy with minimal deleterious effect on the host. Conventional cancer treatment modalities, i.e., chemotherapy, surgery, and irradiation, result in marked suppression of host immune factors. Immune modulating agents when applied judiciously with conventional cytoreductive therapy results in a protective or stimulatory effect on host immune compartments.

Immune adjuvant therapy shown to be effective in both *in vivo* and *in vitro* tumor systems and considered of potential use for supportive therapy in human cancer, is recommended for clinical trial.

Methods Employed:

A. Virus and Tumor Animal Systems

Leukemia and sarcoma inducing viruses:
Transplantable tumors representative of different tumor types; carcinomas, melanomas, sarcomas, and leukemias, are utilized.

B. Immune Response

Humoral and cellular immune responses are measured by standard procedures or by new methods developed by the section staff. Three different assays have been developed to measure macrophage tumoricidal function (a quantitative hemocytometric method, ^{51}chromate release assay and inhibition of DNA synthesis). T-cells are measured by immunofluorescence assay employing θ-antigen antibody; B-cells are measured by immunofluorescence employing antibody to immunoglobulins after column separation of T and B cells. Cellular immune responses are also quantitated by skin graft or allograft systems. Mitogens are also employed *in vitro* to differentiate T and B cell responses.

Major Findings:

Of several chemical and biological agents tested for immune modulating activity some have demonstrated a marked correlation with their capacity to enhance antitumor resistance in the host and their ability to activate

macrophages in vitro. The most notable of these are pyran copolymer, glucan, and BCG. Of particular interest is that histopathological studies show that pyran administered intraperitonealy not only increases macrophage function, but also mobilizes macrophages to migrate to the primary and disseminated tumor sites. In contrast, glucan and BCG must be applied directly near the tumor site to activate macrophage migration and reactivity. The fact that pyran administered systemically causes macrophage mobilization and migration to a tumor at a distal site is important since macrophage tumoricidal function requires cell-to-cell contact. We found no evidence of macrophage migration occurring with glucan or BCG. Direct cell-to-cell contact was established by exposing tumor cells to armed-activated macrophages in vitro and assaying the tumor cell-macrophage mixture in vivo. This passive transfer study demonstrated that the armed-activated macrophages were tumoricidal on contact with tumor cells. Neither activated nor armed macrophages alone exerted a tumoricidal effect.

Of particular significance is the demonstration that mouse interferon directly activates macrophages in vitro to exert a tumoricidal effect on tumor cells. This is important because several of the chemicals and biologicals exerting macrophage activating capability are also interferon inducers.

Pyran copolymer appears to be one of the most potent agents capable of exerting antitumor and immune modulating activity. Results of a study conducted with C^{14} labeled pyran copolymer show pyran is concentrated mainly in the liver and spleen, tissues which are heavily populated with reticuloendothelial and lymphocytic cellular elements. Employing biochemical procedures pyran copolymer was found to derepress cellular DNA synthesis which may account for the larger number of macrophages and other lymphoid cellular elements observed in pyran treated animals. Macrophages were shown to have a high affinity for the C^{14} pyran reaching a saturation point within 24 hours. Of the cellular components examined the nuclear fraction accounts for 35% of the total TCA precipitable radioactivity. Other polyanions were found to compete strongly for TCA precipitable pyran binding sites with poly I:C, dextran sulfate and unlabeled pyran being the most notable competitors.

Of the other immunomodulating agents tested, levamisole and thymosin appear to stimulate lymphocyte replication, specifically T-cell. Both levamisole and thymosin when used in concert with chemotherapy of a murine lymphocytic leukemia resulted in an increased number of mice cured of their disease.

Pyran copolymer was found to be equal to or better than Freunds complete adjuvant when used as an adjuvant with irradiated tumor cell vaccines.

Studies testing immune adjuvant effect on development of metastasis show that after surgical removal of the primary tumor and treatment with either pyran, levamisole, or glucan, a marked decrease of lung metastasis occurred.

Significance to Biomedical Research and the Program of the Institute:

The multidisciplinary approach represented by this project is almost entirely directed towards the prevention, treatment, and control of cancer. Studies with combined immunotherapeutic modalities have led to a more effective control of neoplasias without an additional burden to the host beyond that imposed by chemotherapy. The advantages of such combined modes of treatment are: (1) no additional cytoreductive chemotherapy is needed to maintain remission, (2) the immune competence of the host is restored allowing host immune mechanisms to function more effectively, (3) maintenance of remission may be retained by intermittent treatment with immunoadjuvants which lack the limitations inherent with continuous cytoreductive chemotherapy, (4) refractoriness to immunoadjuvants, if it does develop, can be supplemented by additional chemotherapy with the advantage that the host is in a better physical condition to receive subsequent chemotherapy.

Experimental results conducted with levamisole in this laboratory have led to the inclusion of this agent in chemotherapeutic, surgical and irradiation protocols developed for the treatment of breast Ca, colon and rectal Ca, malignant melanoma, head and neck tumors and leukemias. This section is, and will continue, to collaborate with participating cancer treatment centers requesting information concerning levamisole.

The ability of several immune modulators to specifically and strongly augment host immunity when they are used alone or in concert with established cancer treatment modalities is of a practical value in preventing and/or controlling cancer. Basic research studies conducted with these immunoregulatory agents are defining the cellular components which are activated by these agents and the specificity of their tumoricidal activity. The use of pyran copolymer, thymosin, glucan and interferon is being recommended for clinical trial through the Division of Cancer Treatment.

The ability of the adjuvants to specifically and strongly augment antitumor immunity and isologous tumors after vaccination is of practical value. Of particular significance will be if soluble tumor cell antigens, rather than irradiated tumor cells, can be used for vaccination in concert with the chemical adjuvants. This preventative approach will be applicable to groups at high risk for specific tumor types.

Each of the areas described under Objectives will be pursued. Emphasis will be placed on quantitative and qualitative studies in three specific areas, i.e., immunostimulating adjuvants, combined treatment modalities, and tumor and virus vaccine studies. The role of immunoadjuvants in protracting the remission period induced by chemotherapy in leukemias and surgery in solid tumors will be investigated in detail. Additional in vitro and in vivo methods will be implemented to further define the mechanism of action of the active chemical and biological immunostimulators in terms of T-cell, B-cell, or macrophage function.

Since the macrophage is emerging as an important tumoricidal cell, studies will be conducted to determine the mechanism(s) of macrophage activation and biochemical changes occurring in these activated cells (i.e. intra- and extracellular levels of lysozyme, cytoplasmic LDH, lysozomal enzymes, etc.). Studies will be conducted to determine the role of cyclic nucleotides and prostaglandins in the conversion of the resting macrophage, T or B-cell, to an activated state.

Publications:

Mohr, S.J., Chirigos, M.A., Smith, G.T., and Fuhrman, F.S.: Specific potentiation of L1210 vaccine by pyran copolymer. Cancer Research 36: 2035-2039, 1976.

Schultz, R.M., Papamatheakis, J.D., Stylos, W.A., and Chirigos, M.A.: Augmentation of specific macrophage-mediated cytotoxicity: correlation with agents which enhance antitumor resistance. Cellular Immunology 25: 309-316, 1976.

Schultz, R.M., Papamatheakis, J.D., Luetzeler, J., Ruiz, P., and Chirigos, M.A.: Macrophage involvement in the protective effect of pyran copolymer against the Madison lung carcinoma (M109). Cancer Research 37: 358-364, 1977.

Schultz, R.M., Ruiz, P., Chirigos, M.A., Heine, U., and Nelson-Rees, W.: Establishment and characterization of a cell line derived from a spontaneous murine lung carcinoma (M109). In Vitro (in press, 1977).

Schultz, R.M., Papamatheakis, J.D., and Chirigos, M.A.: Direct activation in vitro of mouse peritoneal macrophages by pyran copolymer (NSC-46015). Cellular Immunology 29: 55-61, 1977.

Mohr, S.J., and Chirigos, M.A.: Potentiation of a tumor cell vaccine by pyran copolymer. In Chirigos, M.A. (Ed.): Progress in Cancer Research and Therapy, Control of Neoplasia by Modulation of the Immune System. New York, NY, Raven Press, 1977, Volume No. 2, p 421-435.

Schultz, R.M., Chirigos, M.A., Mohr, S.J., and Woods, W.A.: Tumoricidal effect in vitro of peritoneal macrophages from mice treated with pyran copolymer. In Chirigos, M.A. (Ed.): Progress in Cancer Research and Therapy, Control of Neoplasia by Modulation of the Immune System. New York, NY, Raven Press, 1977, Volume No. 2, p 437-448.

Schultz, R.M., Papamatheakis, J.D., and Chirigos, M.A.: Interferon the ultimate inducer of macrophage activation by polyanions. Science (in press, 1977).

Price, P.J., Suk, W.A., Skeen, P.C., Chirigos, M.A., Spahn, G.J., and Huebner, R.J.: Geldanamycin inhibition of 3-methylcholanthrene induced rat embryo cell transformation. Proc. Soc. Exptl. Biol. & Med. (in press, 1977).

Books Edited:

First International Symposium, Modulation of Host Immune Resistance in the Prevention or Treatment of Induced Neoplasias. Edited by M. A. Chirigos (1974). GPO Fogarty International Center Proceedings No. 28, 1977.

Second International Symposium, Control of Neoplasia by Modulation of the Immune System, Progress in Cancer Research and Therapy. New York, NY, Raven Press, 1977, Volume No. 2.

Third International Symposium, Control of Neoplasia by Modulation of the Immune System, Progress in Cancer Research and Therapy. New York, NY, Raven Press, 1977, Volume No. 5.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP-04878-08-LRTV

PERIOD COVERED

July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Combined Chemotherapy, Surgery and/or Immunotherapy Against Guinea Pig Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.W. Pearson	Head, Viral Immunotherapy Section	LRTV NCI
OTHER:	D. Klein	Expert	LRTV NCI
	J. Dahlberg	Senior Fellow	LRTV NCI

COOPERATING UNITS (if any)

M. Chirigos, LRTV, NCI
S. Chaparas, DBP, BB
J. Hunter, BGY, NCI

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION

Viral Immunotherapy Section

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two new spontaneous guinea pig models, the L76 leukemia and mammary carcinoma, are under investigation in support of the common antigen and immunotherapy studies. The L76 leukemia exhibited a striking hematological and pathological similarity to acute monocytic leukemia in man. Treatment of leukemic pigs with either one administration of 100 mg/kg of cytoxan or 2 courses of the drug at lower doses over a 2 week period gave a similar suppression of the disease for a 5 week period before relapse and death of 98% of the treated animals. Surgical removal of the spontaneous mammary tumor (15-20 mm) alone or followed by cytoxan therapy resulted in metastasis only to the lungs. In vitro and in vivo testing for drug suppression against the L_2C and L76 leukemias has shown the animals to be immunologically competent one week after drug therapy. A combined modality approach utilizing chemotherapy, surgery, immune-stimulators (C. parvum and glucan) and vaccines alone or in combination is being applied to the L_2C and L76 leukemias and the spontaneous mammary carcinoma in guinea pigs.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Over the past year, the aim of the project has been to develop and evaluate effective therapeutic control of spontaneous, chemical and viral-induced tumors in various strains of guinea pigs under controlled conditions. The therapeutic measures encompass drug therapy, surgery, vaccines and non-specific immune stimulators used alone or in combination against the above mentioned transplantable tumor lines. In addition, in vitro techniques have been employed to monitor the immune response of animals following various forms of therapy to determine fluctuations in the tumor response of treated animals. Any therapy leading to effective control will be submitted for preclinical testing, evaluation and eventual clinical trial.

Methods Employed:

1. Transplantable virus-associated Gross leukemia in strain 2 guinea pigs for in vivo and in vitro studies.
2. Transplantable chemically induced sarcoma in strain 13 guinea pigs for in vivo and in vitro studies.
3. Spontaneous transplantable leukemia in strain 2 guinea pigs for in vivo and in vitro studies.
4. Spontaneous transplantable mammary carcinoma in strain 2 guinea pigs for in vivo and in vitro studies.
5. Transplantable virus-associated Kirsten sarcoma in strain 13 guinea pigs for in vivo and in vitro studies.
6. Transplantable mammary carcinoma in female Fischer rats for in vivo and in vitro studies.
7. Surgical techniques are being applied to remove the primary tumor at the initial site of inoculation.
8. The application of chemotherapy for control of metastatic lesions and/or leukemic remission.
9. Migration inhibition (MIF) and ^{51}Cr release cytotoxicity assays are used to monitor the cell-mediated response of pre-sensitized animals following drug suppression and/or immunotherapy.
10. Soluble tumor-specific antigens (TSA) are being prepared by (3M) KCL extraction procedures to monitor delayed-type cutaneous hypersensitivity responses in guinea pig tumor systems.

Major Findings:

Approximately six months ago, a new spontaneous leukemia (L76) and a mammary carcinoma in strain 2 guinea pigs were discovered and are currently serving as model systems in support of the ongoing common antigen and chemo-immunostimulation investigations. Preliminary histopathological studies revealed that the L76 leukemia exhibited a striking hemotological and pathological similarity to acute monocytic leukemia occurring in man. This observation is in contrast to the L_2C strain of guinea pig (Gross) leukemia which was found to be identical to acute lymphoblastic leukemia occurring in man. Autopsies performed on animals bearing the L76 leukemia near death revealed generalized leukemia characterized by massive metastatic involvement of lymph nodes, liver, spleen, lungs, kidneys, intestines and bone marrow. Hematologically, the WBC counts ranged between 80,000 to 120,000 per mm^3 in animals before succumbing to the leukemia. Treatment of leukemic animals with one course of 100 mg/kg of cytoxan or with cytoxan (50 mg/kg) followed a week later by 25 mg/kg of the drug resulted in suppression of the leukemia (remission period) for approximatley 5 weeks before relapse and eventual death of 98% of the drug treated animals. The period of "remission" was characterized by disappearance of the tumor mass at the site of inoculation, a decrease in the degree of organ involvement and a normal peripheral blood picture. At "relapse", the drug treated animals exhibited increased WBC counts and reappearance of blast cells in the peripheral blood, however, no meningeal leukemia was observed as reported with cytoxan treated animals bearing the L_2C leukemia (Gross) at the time of relapse. Treatment of L76 infected animals with 50 mg/kg of cytoxan followed a week later with 2 or 1 mg/kg of MECCNU gave a similar response as described for cytoxan therapy. Immunological (Dr. Klein) and virological (Dr. Dahlberg) studies involving the L76 leukemia will be discussed in their reports.

Inoculation of strain 2 female guinea pigs subcutaneously (S.C.) or intradermally (I.D.) with the spontaneous mammary carcinoma tumor revealed that the tumor slowly evolved at the site of inoculation with eventual metastasis to the lungs of all infected pigs. Histological examination of all other internal organs were found to be negative for tumor. The infected animals succumb to the disease within 60 to 80 days. Interestingly, tumor bearing animals (15 to 20 mm's) that underwent surgery for removal of the primary tumor or were subjected to surgery followed by various doses of cytoxan therapy revealed a similar metastatic involvement of the lungs with all other internal organs free of tumor 100 days after tumor cell inoculation. Animals are now being held, regardless of the above mentioned treatments, for death. It is hoped in the near future that this spontaneous mammary carcinoma will serve as a model for a combined approach of surgery, chemo-therapy and immunotherapy in the control of the metastatic lesions occurring in the lungs. At the moment, the treated animals have shown no physical signs of brain involvement. Electron microscopic examinations have revealed no virus particles in the primary tumor. This model system is also being used in the common antigen studies and these results will be discussed by

Dr. Klein in his report. A similar modality approach is underway utilizing a chemically induced sarcoma in strain 13 guinea pigs. Preliminary histopathological observations have revealed tumor metastasis in the liver following S.C. inoculation of the tumor cells.

Over the past year, chemo-immunostimulation therapy studies have continued in hopes of controlling the virus-associated Gross leukemia in strain 2 guinea pigs. Intensive cytoxan therapy over a two week period has induced a "remission period" of 5 to 6 weeks with eventual development of meningeal leukemia and death. In vivo and in vitro studies utilizing DNCB, BCG and various mitogens have shown that the cytoxan treated animals were immunologically competent one week after the second course of cytoxan therapy and remained in this state over a period of 3 weeks. To parallel with the above mentioned drug studies, investigations have been underway to analyze the effect of 2 non-specific immune stimulations alone against the L_2C leukemia. The two stimulators, glucan and C. parvum, have been employed one day after tumor cell inoculation and administered as a single shot or as multiple injections. The administration of C. parvum at a dose of .25 mg via the I.A.-S.C. route one day after an S.C. inoculation of 1×10^4 L_2C blast cells was found to increase the MST of the treated animals to 36 days as compared to a MST of 21 days in the untreated group. Multiple injections of C. parvum at the same dose and route was shown to be ineffective. Other routes of injection, i.e., I.P., S.C., I.M. of C. parvum at the same dose level or different doses, both high and low, were without effect and in some cases enhancement of the leukemia was observed leading to early death of the animals. In contrast, multiple injections of glucan via the I.P. route given on a mg/kg basis was found to be more beneficial to the animals than a single administration. Studies have shown that 2 mg/kg of glucan given one day after L_2C blast cell inoculation (1×10^4) followed by the same dose every 4 days for a total of 5 treatments increased the MST to 33 days as compared to a 21 day MST in the untreated group. A single injection of glucan (2mg/kg) I.P. was found to be ineffective. Likewise, single or multiple injections of glucan at different doses via either the S.C., I.M. or I.V. routes had no effect against this leukemia. Studies are currently underway where x-irradiated L_2C blast cells at different doses are being mixed with either C. parvum (.25mg) or glucan (2mg/kg) and subsequently administered by the best regimen and route as described for C. parvum and glucan when used alone against this leukemia. It is hoped a synergistic effect will be achieved against this leukemia. If so, the stimulators in combination with the x-irradiated L_2C blast cells will be used in combination with drug therapy against the L_2C leukemia.

In support of the common antigen studies (Dr. Klein's report) attempts have been going on to develop a virus-induced neoplasia in guinea pigs. Sera samples obtained from pigs inoculated at 24 hours of birth followed by weekly injections for 2 months with either Moloney sarcoma, Rauscher or SV_{40} viruses have failed to show any antibody via CF tests. Likewise, no gross signs of disease have been detected in the virus inoculated animals. Recently, Moloney sarcoma and Rauscher viruses were inoculated intrautero

in hopes of inducing neoplasia. Preliminary CF testing has shown no antibody levels against the 2 viruses. These animals are being continuously monitored for the presence of antibody and any physical signs of disease.

Significance to Biomedical Research and the Program of the Institute:

One aspect of the current program within the Viral Immunotherapy Section is directed toward the treatment and control of spontaneous, chemical and virus-induced leukemias, carcinomas and sarcomas in guinea pigs. Comparisons made by the use of drugs or surgery alone or in combination with immune stimulators and vaccines may lead to more effective measures.

Proposed Course:

Investigations within the Viral Immunotherapy Section are focused on new conceptual and technical approaches in the study of virus cell-host interrelationships (Dahlberg), immunological aspects (Klein) and/or the prevention and/or control (Pearson) of neoplasia utilizing the guinea pig as a model system. A combined modality approach utilizing chemotherapy, surgery, immune stimulators, and vaccines alone or in combination against the L_2C and L76 guinea pig leukemias, a spontaneous mammary carcinoma and a chemically induced sarcoma in guinea pigs will continue over the next year. Studies will continue to develop a viral-induced neoplasia in guinea pigs in support of the common antigen studies.

Publications:

Michalides, R., Schlom, J., Pearson, J.W., and Dahlberg, J.: Characterization of the oncornavirus particles in the plasma of guinea pigs with the L_2C leukemia. J. Virol. 18: 1120-1130, 1976.

Klein, D. and Pearson, J.W.: Modulation of host immune response against the L_2C guinea pig leukemia. Fed. Proc. (In press, 1977).

Schlom, J., Michalides, R., Perk, J., Pearson, J.W. and Dahlberg, J.: Biochemical properties of the B-type retravirus of guinea pig and an agent in the plasma of guinea pigs with L_2C leukemia. Fed. Proc. (In press, 1977).

Perk, J. and Pearson, J.W.: Therapeutic trial of an animal analog for meningeal leukemia with MECCNU. Israel J. Med. Sciences (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04922-11-LRTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Type C RNA Virogenes and Oncogenes in Experimental Animal and Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.J. Huebner	Chief, Lab. RNA Tumor Viruses	LRTV	NCI
OTHER:	P. Arnstein	Veterinary Director	LRTV	NCI
	R. Hill	Biol. Lab. Tech.	LRTV	NCI
	P. Hill	Supv. Bio. Lab. Tech.	LRTV	NCI
	R. Trimmer	Bio. Lab. Tech.	LRTV	NCI

COOPERATING UNITS (if any) S. Aaronson, LRTV,NCI; J. Stephenson, LRTV,NCI; G. Kelloff, LRTV,NCI; M. Kende, LRTV,NCI; D. Fish, FCRC; J. Gruber, VO,NCI; J. Hartley, NIAID; M. Gardner, USC; R. McAllister, USC; P. Price, MAI; J. Rhim, MAI; M. Vernon, MAI; B. Sass, MAI; R. Gilden, FCRC; F. Dixon, Scripps; R. Lerner, Scripps

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program

SECTION
Laboratory of RNA Tumor Viruses

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	1.5	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Prevention and/or suppression of cancer was achieved in several different mouse systems. Passive antibodies (IgG), active killed and live vaccines in several repetitive experiments successfully prevented leukemia ($P < .001$) in AKR mice and prevented 3MC induced sarcomas in C3H/f mice ($P < .006$). Although the protection offered (IgG) is easily explained by long term suppression of AKR virus (Akv-1 and Akv-2 genes) and suppression of the AKR recombinant oncogene, the mechanism of suppression of sarcomas in C3H/f mice which are free of virus expression is still to be determined. It appears in both instances that high titered circulating specific antiviral antibodies are sufficient to prevent the oncogenic rearrangement of the cell membranes. We obtained evidence in the C3H/f system which suggests that IgG having high titers to both ecotropic and xenotropic viruses (and possibly a recombinant) was responsible for protection. A common tumor antigen shared by all known rat tumors including KiMSV(NP) sarcogene transformed cells provided a transplantation cell mediated rejection system in which the KiMSV(NP) rat tumor cell not only rejects itself but also spontaneous lymphoma, 4NQO induced sarcomas, DMH induced colon cancers and polyoma induced tumors of the rat.

Project Description

Objectives:

1. Studies of prevention of spontaneous and induced cancer in mice and rats with passive, active and transplantation specific immunity. The rat tumor prevention experiments already completed provide a virus free model for developing concepts and strategies for prevention of human cancer.

2. To study the mechanisms by which cancer can be prevented (and caused) in animals (rats and hamsters) which serve as models for projected future studies in primates (chimpanzee and baboon). We will attempt to produce predictable cancers within a year or two using a combination of chemical carcinogens, radiation and immune suppression. When a predictable primate cancer model becomes available, studies of prevention of the cancers will be feasible.

3. Delineation and characterization of common human tumor antigens represent the No. 1 target in LRTV. This goal will be pursued in heterotransplant rat systems following protocols similar to those used previously to identify the tumor antigens of DNA virus transformation and the common rat tumor antigens we already have in hand.

Methods Employed:

Vaccines and IgG's produced at several laboratories funded by VOP's resource group (Dr. Jack Gruber). Facilities and support are chiefly provided at FCRC for animal experimentation.

Major Findings:

1. Specific immunological approaches provided protection against spontaneous and chemically-induced cancers in mice and rats. The procedures included killed and attenuated active anti-oncornavirus vaccines, passive antibodies (IgG) to recombinant murine oncornaviruses, and, induction of transplantation immunity to rat tumor cells in rats immunized with virus free KiMSV(NP) (sarcogene) transformed cells.

2. Killed vaccines given to pregnant mothers followed by a live MSV(GLV) challenge in 10-day-old progeny suppressed type C RNA tumor virus replication and significantly prevented ($P < .003$) spontaneous leukemias in C57L x AKR F_1 mice 450-550 days of age.

3. Passive immune gamma globulin (IgG) vs. RadLV given to neonatal AKR mice prevented spontaneous leukemias ($P < 0.001$) in 3 identical experiments.

4. Passive RadLV IgG given 3 times to weanling C3H/f mice prior to and shortly after 3MC (150 μgm) significantly reduced ($P < 0.006$) the expected high rates (80-90%) of chemically-induced tumors in controls. The protective

immunity was dependent on RadLV recombinant virus used for producing the IgG having equal antibody titers to both the ecotropic RadLV and xenotropic viruses.

5. Transplantable (lethal) rat tumors and tumor cells produced by a variety of chemicals and viruses and containing a common rat tumor antigen were rejected when the rats were previously immunized by KiMSV(NP) sarcogene positive rat tumor cells, which also carry the common rat tumor antigen.

The implications of these tumor prevention experiments, particularly those in the rat, that do not require virogene(s) as immunogen represent perhaps the best models available on which to build feasible approaches to protection against human cancer.

Significance to Biomedical Research and the Program of the Institute:

Prevention of cancer in mice and rats, precisely those animals most used for defining genetic and chemically induced cancer, provide opportunities for identifying mechanisms and methods with which to achieve the specific immunity that must eventually be used in attempts to prevent and/or to modify human cancer.

Proposed Course:

(a) To pursue and employ studies of cancer prevention procedures which are successful in experimental animals; (b) to study the mechanisms responsible for protection; and (c) to develop acceptable immunization procedures which hold promise for success in attempts to prevent cancer in humans.

Publications:

Arnstein, P., Riggs, J.L., Oshiro, L.S. Huebner, R.J. and Lennette, E.H.: Induction of lymphoma and associated xenotropic type C virus in C57L mice by whole-body irradiation. J. Natl. Cancer Inst. 57: 1085-1089, 1976.

Cho, H.Y., Cutchins, E.C., Rhim, J.S. and Huebner, R.J.: Revertants of human cells transformed by murine sarcoma virus. Science 194: 951-953, 1976.

Gardner, M.B., Klement, V., Henderson, B.E., Estes, J.D., Dougherty, M., Casagrande, J. and Huebner, R.J.: Efforts to control type C virus expression in wild mice. In Chirigos, M.A. (ed.): Control of Neoplasia by Modulation of the Immune System. New York, Raven Press, 1977, pp. 391-407.

Gardner, M.B., Klement, V., Rasheed, S., Rongey, R.W., Brown, J.C., Pike, M., Henderson, B.E. and Huebner, R.J.: The pathogenesis of lymphoma and paralysis in wild mice. In Clemmensen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research 1975, Bibl. Haemat., No. 43. Basel, Karger, 1976, pp. 204-208.

Gardner, M.B., Klement, V., Rongey, R.R., McConahey, P., Estes, J.D. and Huebner, R.J.: Type C virus expression in lymphoma-paralysis-prone wild mice. J. Natl. Cancer Inst. 57: 585-590, 1976.

Heiniger, H.J., Huebner, R.J. and Meier, H.: Effect of allelic substitutions at the hairless locus on endogenous ecotropic murine leukemia virus titers and leukemogenesis. J. Natl. Cancer Inst. 57: 1073-1074, 1976.

Huebner, R.J.: The endogenous origin and transmission of RNA viral genomes that code for cancer. In Grundmann, E. (Ed.): Malignant Bone Tumors. Recent Results in Cancer Research Vol. 54. Berlin, Springer-Verlag, 1976, pp. 63-91.

Huebner, R.J., Gilden, R.V., Lane, W.T., Trimmer, R.W. and Hill, P.R.: Suppression of endogenous type C RNA virogene expression in mice by serotype-specific viral vaccines; progress report. In Chirigos, M.A. (Ed.): Control of Neoplasia by Modulation of the Immune System. New York, Raven Press, 1977, pp. 381-389.

Huebner, R.J., Gilden, R.V., Toni, R., Hill, P.R. and Trimmer, R.W.: Suppression of endogenous type C murine virogene expressions with virus specific hyperimmune immunoglobulin (IgG). Proc. of Third Int. Symp. on Detection and Prevention of Cancer. New York, Marcel Dekker, Inc., 1976, in press.

Huebner, R.J., Gilden, R.V., Toni, R., Hill, R.W., Trimmer, R.W., Fish, D.C. and Sass, B.: Prevention of spontaneous leukemia in AKR mice by type-specific immunosuppression of endogenous ecotropic virogenes. Proc. Natl. Acad. Sci. 73: 4633-4635, 1976.

Lee, J.C., Ihle, J.N. and Huebner, R.: The humoral immune response of NIH Swiss and SWR/J mice to vaccination with formalinized AKR or Gross murine leukemia virus. Proc. Natl. Acad. Sci. 74: 343-347, 1977.

Price, P.J., Suk, W.A., Peters, R.L., Gilden, R.V. and Huebner, R,J.: Chemical transformation of rat cells infected with xenotropic type C RNA virus and its suppression by virus-specific antiserum. Proc. Natl. Acad. Sci. 74: 579-581, 1977.

Rhim, J., Cho, H.Y., Huebner, R.J. and Gilden, R.V.: Characterization of Kirsten sarcoma virus transformation of human cells and isolation of non-producer human cells. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research 1975, Bibl. Haemat., 43. Basel, Karger, 1976, pp. 84-87.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04924-04-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Immunoprevention of Spontaneous Tumors with Viral and Cellular Vaccines

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G. J. Kelloff	Head, Viral Immunology Section	LRTV NCI
OTHER:	M. Kende	Expert	LRTV NCI

COOPERATING UNITS (if any)
B. Sass, Microbiological Associates
I. Al-Ghazzouli, Microbiological Associates
R. Peters, Microbiological Associates
R. Donahoe, Microbiological Associates

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Viral Immunology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project has the long range goal of immunoprevention of spontaneous neoplasms in animal model systems that have viral and epidemiological similarities to human neoplasia. To this end, past efforts have included a definition of the endogenous type C viruses and spontaneous tumor expressions in large holding colonies of untreated aging mice and more recently, the project has developed a number of immunoassays to measure the host's natural and induced immune responses to type C viruses and tumor cells, including cellular and humoral cytotoxicity, footpad, lymphocyte transformation, and radioimmunoassays. This project has found that noninfectious viral and virus containing cellular vaccines can be produced which evoke measurable immune responses in the animal and also result in resistance and protection of the animal to challenge with high doses of leukemogenic virus and tumor cells. Studies of the animal's immune response potential versus aging and his endogenous ecotropic and xenotropic type C virus expression (in his tumor cells and his immune system) will provide information to reach the stated goals. The necessary immunoassay and viral assay techniques are now in hand to sort out this complex issue in natural tumor systems and establish the feasibility of these future studies and achievement of the long range goals.

Project Description

Objectives:

(A) Development and utilization of assays for monitoring cell-mediated and humoral immunity in the mouse to type C virus and type C viral antigens and nonviral antigens in cellular vaccines prepared from syngeneic tumor cell lines.

(B) Determination of whether type C virus vaccines or virus containing cellular vaccines can prevent spontaneous, chemically induced, or irradiation induced tumors in mice known to have measurable humoral and cellular immune responsiveness to the viral immunogens.

(C) Determination of the immune competence of laboratory animals with aging and with development of naturally occurring neoplasms and how this relates to endogenous ecotropic and xenotropic virus expression.

Methods Employed and Major Findings:

This effort has focused its major emphasis on the immuno-prevention of spontaneous neoplasms in animal model systems that have viral and epidemiological similarities to human neoplasia. To this end, past efforts have included a definition of the ecotropic type C virus and spontaneous tumor expression in large holding colonies of untreated, aging mice chosen to serve as specific model systems. More recently, the contract has developed and defined a number of immunoassays to measure the host's natural and induced immune responses to type C viruses and its subunits. These assays have allowed the measurement of immune responses to type C viruses and type C virus containing tumor cells after immunization with viral and virus containing cellular vaccines. In the past year, several major studies that are part of an integrated vaccine program have been completed. These include: a study showing reduction of X-ray induced leukemias in C57BL mice by induction of active immunity or transfer of passive immunity to endogenous type C virus. Active immunity was established by immunization with inactivated R-MuLV and passive immunity by transfer of goat anti-G-MuLV immune IgG. Both regimens resulted in measurable circulating antibody to AKR-gp70 throughout part of the latent period of tumor development, both resulted in reduced tumor incidence compared to several controls, and both resulted in apparent immunoselection against virus positive tumors in the tumors that occurred in these groups; a study showing that active immunity can be induced in the BALB/c mouse by banded type C virus vaccine that is adequate to confer resistance to exogenous virus challenge and adequate to suppress and eradicate endogenous type C virus in the spleens of these animals. Both Rauscher leukemia virus and Gross leukemia virus vaccines were effective and both were also shown to induce cell-mediated immunity, and low level neutralizing antibodies. Purified gp70 was also shown to be capable of inducing resistance to challenge with Rauscher leukemia virus; a study showing resistance to virus-free syngeneic tumor transplantation in the BALB/c mouse after immunization with

type C virus infected homologous tumor cells. This study establishes for the first time that syngeneic tumor cell lines derived from spontaneously occurring tumors and shown to be free of endogenous type C virus or its antigens can be effectively immunized against. The effective immunogen is the homologous tumor cells, highly infected with Rauscher leukemia virus, which has been shown to be immunogenic in the adult mouse resulting in no tumor production nor leukemogenesis in the immunized animal. Splenic lymphocytes from the immune animal were shown to be cytotoxic in vitro, and by adoptive transfer, experiments were capable of tumor prevention in vivo; a study of the type C virus structural antigens on the surface of the infected cell and the virus using antisera made by Dr. Stephen Oroszlan against the major structural protein of R-MuLV cell lines infected with ecotropic murine leukemia viruses of the FMR and AKR types and the two known classes of xenotropic murine leukemia virus, Class II and Class III, were examined by a complement dependent cytotoxicity assay. Virus specific cytolytic activity was found with anti-R-gp70 and with anti-R-p12. The activity with the latter sera was not well correlated with the virus content or the virus producing ability of different R-MuLV shedding cell lines whereas activity with gp70 antisera was. Immunoassays of the virion showed that gp70 and p15(E) were on the viral surface whereas p12 and p30 were not; a study showing that two low incidence strains of mice, the BALB/c and the NIH Swiss mouse, have a ubiquitous presence in their spontaneous neoplasms of either ecotropic or xenotropic viral antigens in the former and xenotropic viral antigens in the latter. This is the first publication that NIH Swiss mice have any spontaneous neoplasms, as previous studies have not included observations of old age animals. Of interest is that the spontaneous neoplasms have the p12 antigen of the xenotropic virus, and as has been presented in this report, monospecific antisera to the p12 antigen has shown this antigen to be on the cell surface and can serve as a target antigen in virus infected cells for antibody mediated cell lysis.

The finding of type C viruses or viral antigens in the spontaneous neoplasms of virtually all species where it has been sought has suggested that these viruses are the specific etiological agents of these neoplasms. The demonstration of the oncogenicity of most of these virus isolates provides further evidence for this. Regardless of this etiological role, however, of more practical concern is the fact that the virus is present in these neoplasms and the fact that immunizing preparations of the virus might be effective in attempts at immune prevention of these tumors. In the above mentioned studies it was established that viral antigens were ubiquitous in the tumors in at least two strains of mice. It was felt, therefore, that a cytotoxic or cell killing immune response directed against viral antigens in target cells might provide the means to prevent spontaneously occurring neoplasms, regardless of the etiologic role of the endogenous virus. We have, therefore, established cell-mediated and humoral chromium release cytotoxicity assays. We have found that virus vaccine immunization or virus containing cellular vaccine immunization can result in a cytolytic immune response against target cells containing type C viruses. These assays are

currently being examined in a variety of experiments to determine their specificity, and their ability to predict the in vivo immunity of the animal.

Large scale animal experiments, aimed at prevention of spontaneous, chemically induced, or irradiation induced tumors in the BALB/cCr, NIH Swiss and C57BL mice are currently in progress. These experiments involve viral or cellular vaccines, involve animals of various ages, involve measurement of endogenous ecotropic or xenotropic infectious virus or viral antigens, measurement of the animal's natural and induced cellular and humoral immune responses to the virus and virus positive target cells, and finally involve a rigorous histopathologic review to determine the effect of immunization on the spontaneous and induced neoplasms. These experiments range in duration from 6 months to 21 months and offer an excellent opportunity to understand how the complex virus-host relationship and complex immune responses of the animal can be used to prevent spontaneously occurring tumors. These studies have already shown that old animals containing isolatable ecotropic viruses have their spontaneous lymphoreticular neoplasm incidence enhanced when immunized with type C virus or adjuvant, in spite of circulating antibody to type C virus, but young animals when immunized prior to endogenous virus expression have no detectable virus in their spleens when their aged matched controls have up to two logs of isolatable virus.

Significance to Biomedical Research and the Program of the Institute:

This effort has contributed significantly to an understanding of the endogenous type C virus relationship with its host and its genetic and epigenetic relationship to the host's naturally occurring tumors. Many of the necessary immunoassay techniques and virus assay (infectious and antigenic) techniques are now in hand to begin to sort out these complex issues and make immunoprevention of spontaneously occurring and appropriate experimentally induced tumors a realistic goal to pursue.

Proposed Course:

Planned studies include attempts to immunoprevent spontaneously occurring tumors in the BALB/c mouse with viral and cellular vaccines, attempts to immunoprevent chemically induced tumors in the BALB/c and C57BL mouse with cellular vaccines containing type C virus, and further experimentation with irradiation induced tumors in C57BL mice. Other studies will involve attempts to understand the animal's immune response potential versus aging and versus his endogenous virus expression (both ecotropic and xenotropic), rigorous developmental work on cellular and humoral cytotoxicity assays including a definition of the role of the type C virus in specifying the critical target cell antigens, and attempts to understand the complex interaction of humoral and cellular immunity and their respective roles in determining spontaneous neoplasia incidence.

Publications:

Kelloff, G.J., Peters, R.L., Donahoe, R.M., Al-Ghazzouli, I., Sass, B., Nims, R.M. and Huebner, R.J.: An approach to type C virus immunoprevention of spontaneously occurring tumors in laboratory mice. Cancer Res. 36: 622-620, 1976.

Donahoe, R.M., Peters, R.L., VanVleck, R., Huebner, R.J. and Kelloff, G.J.: Development of a lymphocyte transformation microassay in the mouse to murine leukemia virus. J. Natl. Cancer Inst. 56: 51-57, 1976.

Kelloff, G.J., Peters, R.L., Donahoe, R.M., Stephenson, J.R. and Aaronson, S.A.: Natural occurrence of tumors in mouse strains differing in their xenotropic and ecotropic endogenous viruses. J. Natl. Cancer Inst. 57: 85-89, 1976.

Sass, B., Peters, R.L. and Kelloff, G.J.: Differences in tumor incidence in two substrains of Claude BALB/c (BALB/cfCd) mice, emphasizing renal, mammary, pancreatic, and synovial tumors. Lab. Animal Sci. 26: 736-741, 1976.

Stephenson, J.R., Peters, R.L., Hino, S., Donahoe, R.M., Long, L.K., Aaronson, S.A. and Kelloff, G.J.: Natural immunity in mice to structural polypeptides of endogenous type C RNA viruses. J. Virol. 19: 890-898, 1976.

Al-Ghazzouli, I.K., Donahoe, R.M., Huang, K-Y., Sass, B., Peters, R.L. and Kelloff, G.J.: Immunity to virus-free syngeneic tumor cell transplantation in the BALB/c mouse after immunization with homologous tumor cells infected with type C virus. J. Immunol. 117: 2239-2248, 1976.

Peters, R.L., Sass, B., Stephenson, J.R., Al-Ghazzouli, I.K., Hino, S., Donahoe, R.M., Kende, M., Aaronsin, S.A. and Kelloff, G.J.: Immunoprevention of X-ray-induced leukemias in the C57BL mouse. Proc. Natl. Acad. Sci. USA, in press.

Kende, M., Sass, B., Donahoe, R.M., Al-Ghazzouli, I.K. and Kelloff, G.J.: Type C virus structural antigens on the surface of the infected cell as determined by a humoral cytotoxicity assay. J. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04925-06-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Type C RNA Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. S. Sarma	Head, Animal Virology & Field Studies Section	LRTV NCI
OTHER:	R. Huebner	Chief	LRTV NCI
	P. Hill	Supv. Biol. Lab. Tech.	LRTV NCI

COOPERATING UNITS (if any) E. Premkumar-Reddy, Micro. Assoc.; J. Gruber, PRL, NCI; D. Howell, PRL, NCI; R. Gallagher, LTCB, NCI; A. Hellman, VOP, NCI; M. Gardner, USC

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION Animal Virology & Field Studies

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	2.0	5.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies were undertaken to determine the prevalence and natural behavior of type C RNA tumor viruses of cat, chicken, mouse, and man. Studies of spontaneous expression of endogenous type C RNA viruses in lymphocyte cultures derived from mice of different strains and humans showed that a spontaneous and predictable release of B-tropic virus occurred in lymphocytes derived from three BALB/c mice of certain age groups. Virus release was not observed in three lymphocyte cultures derived from other mouse strains or humans. The group specific antigen of avian reticuloendotheliosis virus was isolated and characterized. A sensitive radioimmunoassay was developed for the detection of REV antigens and antibodies. The technique of radioimmunoassay was simplified by the use of killed Staphylococci as immunoabsorbant for precipitating antigen-antibody complexes. Feline cells were found to be consistently more sensitive than rat cells to the cell transforming effects of primate sarcoma viruses including the cell-transforming HL23 virus. Repeated tests failed to provide evidence to suggest that human sera of cancer patients or laboratory workers contain viral neutralizing antibodies against the primate sarcoma viruses.

Project Description

Objectives:

This section is concerned with laboratory and field studies on type C RNA tumor viruses to determine their prevalence, natural behavior and modes of transmission in selected mammalian and avian species and establish their role in naturally occurring and experimentally induced cancers.

Major Findings:

Studies on a Type C Virus of Cat: A feline cell culture producing a type C RNA virus, but with characteristics sufficiently different from FeLV or RD-114 viruses was studied. Many of the protocols were designed to examine the questions of the host range of the virus, its reactivity in complement fixation test against standard gs antisera prepared against known type C viruses and its reactivity in the radioimmunoassay technique, specifically against FeLV and RD-114 gs antisera. Both FeLV and the suspected new virus (SNV) are being produced in large scale in an attempt to determine the antigenic (p30, p12) and biochemical (RNA, DNA) interrelationships between the virus, virogenic and virus-producing cells of the FeLV, RD-114 and SNV systems.

Production of pseudotypes of a feline sarcoma virus: In a recent phenotypic mixing experiment we showed that the Snyder-Theilen strain of feline sarcoma virus is defective for the synthesis of viral envelope glycoprotein and thus the virus can be made to accept and retain a new viral envelope by phenotypic mixing. A sarcoma pseudotype with envelope of subgroup C virus was thus prepared. The virus produced sarcomas in guinea pigs. A nonproducer cell line established from a guinea pig tumor was found to contain the sarcoma viral genome. Pseudotypes of feline sarcoma virus were readily prepared by rescue of the sarcoma genome with helper feline leukemia viruses of subgroups A, B and C. These pseudotype viruses appear to be better than MSV(FeLV) pseudotypes as indicator viruses in virus neutralization and viral interference test in that the virus titers are higher and the foci are larger and easier to count.

Comparative sensitivity of cat and rat cells to productive infection and cell transformation by primate type C viruses: In studies of the host range of the suspected human isolate, HL23, we found that the HL23 virus as well as woolly monkey sarcoma virus induce cellular morphologic transformation in feline embryo cells. Our initial observation suggested that cat cells may be more sensitive than rat embryo cells to the cell transforming effects of these viruses. We compared the relative abilities of these viruses and their associated nontransforming viruses to replicate and cause productive infection in these cultures. Present results suggested that cat cells are between 10 to 100 times more sensitive than rat cells to the cell transforming effect of the viruses but there were no noticeable differences between the cells of the two species in their ability to support the replication of these viruses.

Studies on bovine leukemia: We recently developed a sensitive radioimmunoassay technique for the detection of the major group specific antigens and antibodies of the viral agent of bovine leukemia. Experiments are now in progress to confirm recent findings of Ferrer and co-workers on the development of a syncytial in vitro assay for the viral agent of bovine leukemia. A viral neutralization test will be developed for the detection and assay of viral neutralizing antibodies. This test will be used in sero-epidemiological studies to examine the question of prevalence of the virus in bovines and other species and possible exposure of man to this virus.

Spontaneous expression of endogenous type C RNA viruses by BALB/c splenic B-lymphocytes in continuous culture: B-lymphocyte cultures were established from spleens of BALB/c, C57B1/6, NIH Swiss, and SWR mice of various age groups. Spontaneous, consistent and thus predictable release of a B tropic mouse endogenous virus occurred from the very first passage in cultured lymphocytes derived from BALB/c mice six months or older but not from similar lymphocytes derived from BALB/c mice of 1.5 months or 3 months of age. C57B1/6, NIH Swiss and SWR mice belonging to various age groups ranging between 1.5 months and 18 months failed to exhibit such a spontaneous release of viral particles. We conclude that in BALB/c splenic B-lymphocytes, a breakdown of cellular control mechanisms occurs in older animals leading to viral production while such a phenomenon is absent in C57B1/6, NIH Swiss and SWR mice.

Continuous culturing of human splenic B-lymphocytes: We recently described a method for the continuous culturing of murine splenic B-lymphocytes. This method involved the use of rat-tail collagen-coated glass and plastic surfaces which seem to specifically enhance adherence and propagation of splenic B-lymphocytes. This method was extended to human and cat spleens. Several spleens derived from normal and cancer patients were obtained and the cells established in culture. Immunoglobulin biosynthesis was studied and the cells were found to synthesize IgM molecules. All the cell culture supernatants were tested for the presence of type C RNA viral particles using RNA dependent DNA polymerase assays. All cultures, including those derived from cancer patients, were found to be negative. Virus induction studies with halognated pyrimidines are currently in progress.

In vitro transformation of mouse lymphocytes using Abelson virus: Abelson virus stocks were prepared from ANN-1 non-producer cells using Moloney strain of mouse leukemia virus as the helper virus. The virus stocks were examined for infectivity titers by in vitro methods such as XC-test and focus-formation assay. When BALB/c splenic lymphocytes derived fresh from animals were infected with this virus, a transformed cell line was obtained which was negative for Θ-antigen and positive for surface immunoglobulins. The cells, when injected into BALB/c mice produced tumors within a 20-30 day period. Currently, experiments are being carried out to use this technique for the isolation of antigen-specific B-lymphocytic tumors. Such cell lines would be very useful in studying the molecular mechanism of immune stimulation following antigen-antibody receptor reactions on the cell membranes.

A simplified radioimmunoassay for viral antigens: Use of Staphylococcus aureus as an adsorbant for antigen-antibody complexes: A rapid method was developed for the radioimmunoassay of viral antigens using Staphylococcus aureus as the adsorbant for antigen-antibody complexes. The method utilizes the strong affinity of "protein A" molecules present on the cell walls of certain strains of Staphylococci towards IgG molecules. The results show that this method could be used with the same or probably higher sensitivity in the precipitation as well as competition radioimmunoassays for type C viral antigens and antibodies.

Isolation of gs antigen of reticuloendotheliosis virus (REV) and development of a radioimmunoassay for seroepidemiology: REV was produced in quantities suitable for isolation and purification of the major group specific (gs) antigen. This antigen was purified by column chromatographic techniques from disrupted virus. Polyacrylamide gel electrophoretic analysis revealed that the preparation was greater than 95% pure. Using this antigen, we developed a radioimmunoassay using the rabbit antiserum against the purified gs antigen. Currently experiments are in progress to test the cross-reactivity of this antigen with other known avian viral gs antigen. Also, the assay is being used to test the presence of antibodies against this gs antigen in sera of turkeys which are a) normal, b) experimentally infected with REV, and c) naturally infected with REV.

Test to determine prevalence of virus neutralizing serum antibodies in humans against the primate type C virus: We previously found that a small proportion of laboratory workers (1 of 49) and leukemic human subjects (1 of 49) carried factors in their sera which at low serum dilutions (undiluted or 1:2 dilution) neutralized the infectivity of 25 to 100 infectious units of the woolly monkey associated virus (1 of 49 laboratory worker sera) or a similar dose of the HL 23 "Gallo" virus (1 of 49 human leukemic sera). Retest of the same sera at the same dilutions, however, gave negative results suggesting that virus neutralization previously observed may have been due to nonspecific factors.

Significance to Biomedical Research and the Program of the Institute:

Present studies provided important information on the biology, prevalence and natural behavior of the type C RNA tumor viruses of the cat, mouse and man. A recently developed method for continuous culturing of mouse splenic lymphocytes led to studies which provided insights on the occurrence and host cell control of a B-tropic endogenous type C virus of BALB/c mouse. Methods were developed for production and use of pseudotypes of a feline sarcoma virus in viral interference and antibody assays. Cat cells were found to be more sensitive than rat cells to cell transformation effects of primate sarcoma virus. A simplified method was introduced for radioimmunoassay (RIA) of viral antigens and antibodies. A sensitive RIA was developed for the detection of the gs antigens and antibodies of the avian reticuloendotheliosis virus. Our repeated studies have failed to confirm our previous findings that low dilutions of certain human sera neutralize the infectivity of the HL23 virus and the woolly monkey sarcoma virus.

Proposed Course:

Studies will be continued on bovine leukemia for the development and use of standardized procedures for assay of virus and virus neutralizing antibodies. Newly identified type C viruses of the cat will be characterized. The genetic control of the expression of B-tropic virus in BALB/c mice will be studied.

Publications:

Premkumar-Reddy, E., Price, P.J., Chung, K.C. and Sarma, P.S.: Continuous culturing of murine splenic B-lymphocytes: Synthesis and surface deposition of IgM and putative IgD molecules. Cell 8: 397-403, 1976.

Devare, S.G., Stephenson, J.R., Sarma, P.S. and Aaronson, S.A.: Bovine lymphosarcoma: Development of a radioimmunologic technique for detection of the etiologic agent. Science 194: 1428-1430, 1976.

Sarma, P.S. and Log, T.: Defectiveness of a feline sarcoma virus demonstrable by the acquisition of a new envelope antigenic type by phenotypic mixing. Virology, in press.

Premkumar-Reddy, E., Devare, S.G., Vasudev, R. and Sarma, P.S.: A simplified radioimmunoassay for viral antigens: Use of Staphylococcus aureus as an adsorbant for antigen-antibody complexes. J. Nat. Cancer Inst., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04930-06-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biology of Natural and Induced Neoplasias and Their Endogenous Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. Arnstein	Veterinary Director	LRTV NCI
OTHER:	R.J. Huebner	Chief	LRTV NCI
	P. Sarma	Head, Animal Virology & Field Studies Section	LRTV NCI
	G. Todaro	Chief	LVC NCI
	S. Aaronson	Head, Molecular Biology Section	LRTV NCI

COOPERATING UNITS (if any) E.H. Lennette, Calif. Dept. Health; P. Price, Micro. Asso.; M. Gardner, USC; R. Gilden, FCRC: J. Rhim, Micro. Asso.; R. McAllister, Childrens Hosp. of LA: J. Levy, U. Calif.; W. Nelson-Rees, Naval Bio. Res. Lab., Oakland; H. Kaplan, Stanford; M. Lieberman, Stanford

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Viral Immunology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Tumors induced by transplants, viruses, chemicals and radiation are studied in mice. Prevention of these tumors by various vaccines is subsequently tested. Mouse strains of low spontaneous cancer incidence, resembling the human experience are used in most studies. Evidence of xenotropic type C virus has been found in all murine tumors. Vaccines prepared from tumors and cultured cells have produced promising protection from challenge. New methods of producing killed vaccines are compared to the standard formalin inactivation. Experimental xenografting to athymic nude mice (which are hereditarily immunodeficient) continues to prove an excellent measure of cell malignancy and transformation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. Study representative transplantable neoplasms with emphasis on in vivo tumor formation, type C virus activity, chemical and radiation inducibility and establishment in tissue culture.

2. Design, develop and carry out experiments which will measure response to anti-cancer vaccines, especially those containing type C viruses and tumor cell antigens.

3. Study human and other tumor xenografts in immunodeficient animals.

Methods Employed and Major Findings:

I. Oncornavirus biology

A. Experiments on 129J mice

Following confirmation of xenotropic type C virus in C57L mice by two different isolation procedures, transplantation of permissive non-murine tumor cells to immunodepressed mice (Arnstein et al., JNCI 53: 1787, 1974) and radiation lymphoma induction and subsequent cocultivation of lymphoma cells with permissive tumor cells (Arnstein et al., JNCI 57: 1085, 1976), both techniques have been applied in attempts to identify and isolate an oncornavirus in 129J mice. This strain is known to produce no ecotropic type C viruses at any time and the evidence for murine xenotropic type C virus (MuX) is limited to the presence of a glycoprotein (gp70) in selected normal tissues. 129J mice have one of the lowest incidences of lymphoma among inbred mice.

Xenotransplantation of permissive human and canine tumor cells to immunodepressed 129J's following procedures which had yielded NIH Swiss and C57L xenotropes gave negative results: no replicating virus was isolated; there was no induction of viral antigens p30 or gp70 in the receptor lines, even after several serial blind passages and treatment with the potent oncornavirus inducer iododeoxyuridine (IdU) as described in Arnstein et al., JNCI 57: 1085, 1976; and there was no evidence of the enzyme reverse transcriptase (RT), which is characteristic of the oncornaviruses, in cultures reestablished from transplants. Subsequently, irradiation was used and, as expected, proved less lymphomagenic in 129J's than in other strains of mice: only 4 of 15 receiving sublethal, but usually oncogenic, whole body radiation (200 roentgens weekly for 4 weeks) developed lymphomas. The primary tumors and tissue cultures established from them proved negative for type C virus, either directly or by cocultivation with cells permissive for murine xenotropes. Three serial passages of the first lymphomas in suckling 129J's were also

negative for virus; a pool of tumors from the fourth released a typical xenotropic type C virus after cocultivation with the D17 canine cells. A chronically infected canine line has been expanded and now produces $\sim 10^6$ infectious type C particles per ml. The new isolate resembles other MuX's occurring spontaneously, such as BALB3, NZB, C57L and similar isolates, and is antigenically distinct from "inducible" MuX's such as BALB2 (Stephenson, personal communication). Attempts to reisolate 129J MuX from other tumors have been negative. The publication will be delayed until at least a second viral isolate is established.

II. Transplantable neoplasms: propagation and vaccination.

C57L mice

Numerous experiments have been conducted to vaccinate C57L mice, eliciting subsequently demonstrable tumor rejection. The findings are that this strain, although low in natural tumor incidence, reacts very poorly to isologous tumor vaccines. A summary of vaccination results in healthy C57L adults follows: (For the time being, no additional vaccination of C57L's will be carried out.)

A. Vaccines made of lymphoma cells, either inactivated or given live in subtumorigenic numbers, fail to protect from the same lymphoma in the usual challenge dose.

B. An intensive vaccination schedule with living lymphoma cells (5×10^6) given 3 times at weekly intervals into foot pads (C57L lymphoma cells do not initiate lymphomas by this route) failed to protect against the same number of cells inoculated subcutaneously in the abdomen, 2 weeks after the vaccination.

C. C57L sarcoma cells given into footpads produce large, progressive tumors. After amputation of the affected leg, the mouse is fully susceptible to rechallenge with the same sarcoma line in the abdominal subcutis.

D. Repeated vaccination with freeze-killed sarcoma and lymphoma cells fails to protect from challenge with the same line, injected viable.

E. Photoinactivated (UV + psoralen) and formol-killed tumor cells vaccinates fail to protect against the isologous viable challenge.

NIH Swiss mice

This mouse strain has been more promising as a system for testing protection by cellular and viral tumor antigens against grafted or induced neoplasms. The spontaneous fibrosarcoma N275 is especially useful as a challenge after various vaccination schemes.

A. N275. This line of neoplastic cells derived from a spontaneous NIH Swiss fibrosarcoma has been described in the previous annual report. It is negative for oncornavirus and its major antigens (p30 and gp70), tumorigenic for NIH Swiss mice, but non-tumorigenic for other strains. Recently it has been observed that high passages (70 and above) have diminished tumor-inducing capability: where 10^5 viable cells inoculated subcutaneously readily induced progressive, fatal sarcomas in all recipients at P15-40, 10^7 cells were required for the same result by P70. It was also apparent that the culture was composed of two subpopulations: slender fibroblastoid elements growing in random orientation and plumper, more pleomorphic cells. Subsequently, two distinct sublines have been segregated, as follows:

N275 LT (low tumorigenicity) is passaged at regular weekly intervals with trypsinization and dispensing into outgrowth medium with 10% fetal bovine serum. It is mostly made up of slender, criss-crossing cells, produces "regressor" tumors in normal adult NIH Swiss mice, and steadily progressive tumors in athymic "nude" mice.

N275 HT (high tumorigenicity) is passaged by detaching cells mechanically (gentle shaking) and immediately dispensing into fresh medium using 5% FBS. It is a plump, polygonal cell and produces "progressor", ultimately lethal, sarcomas in normal adult NIH Swiss mice and extremely fast growing tumors with frequent metastases in the athymic "nudes". This subline has "returned" the minimal tumorigenic dose to about 10^5 cells.

In the normal adult NIH Swiss mice, pre-inoculation with N275 LT cells protects vaccinates against challenge with N275 HT. The following effects on N275 HT tumorigenicity have been observed:

1. Pre-inoculation with live N275 LT (1 or 2 doses totaling 2×10^6 cells subcut.) give 80-100% protection against up to 5×10^6 HT cells.

2. Formalin or glutaraldehyde-killed N275 cell vaccines in multiple doses reduce tumor mortality about 30% (preliminary data).

3. Vaccine prepared with N275 cells photoinactivated by psoralen and UV light reduces tumor incidence by about 60% (preliminary data). This is somewhat more promising than other killed vaccines.

4. Pre-inoculation with live high passage NIH Swiss 3T3 fibroblasts derived from normal tissue (Jainchill et al., 1969) gives excellent protection, resembling the N275 LT cells (preliminary data). Killed 3T3 cells will be tried next.

5. Live vaccine prepared from non-tumorigenic lymphoid line 34489 originally adapted to tissue culture from NIH Swiss tumor by Dr. J. Hartley reduces N275 HT tumor incidence about 50%. Killed 34489 cells give no protection.

6. Pre-inoculation with 3 doses of fetal bovine serum reduces tumor incidence by 30-40%.

7. Pre-inoculation with Corynebacterium parvum vaccine reduces incidence about 50%.

8. Repeated doses of live xenotropic oncornavirus fail to protect from N275 HT challenge.

We are studying the roles of specific and non-specific antigens in the preparations which afford significant protection.

B. Other transplantable tumors. Two highly malignant lymphoma transplants, ENU 635-45, induced by transplacental ethyl nitrosourea, and N646-48, resulting from chronic antigenic stimulation, are being maintained by mouse → mouse passage. Attempts are in progress to adapt them to tissue culture growth, so that they can be used in reproducible vaccine trials. These tumors produce "takes" in any age NIH Swiss mice (but not in other strains) and do not produce any ecotropic MuLV.

A spontaneous breast carcinoma and chemically-induced lung carcinomas and sarcomas have also been established for future trials.

III. Chemical carcinogenesis

Additional experiments using tumor cell vaccines followed by 3 methylcholanthrene (3MC) challenge confirmed the delaying effects of 3MC tumor onset of repeated doses of subtumorigenic cell implants or unrelated antigens (FBS, Corynebacteria). Reduction of final 3MC tumor incidence could not be demonstrated to date.

IV. Xenotransplantation to athymic nude mice. In collaboration with J. Rhim, we compared in vitro evidence of cellular transformation with a shift from "no take" to "take" in the above immune-deprived host.

Human and canine cells have been selected for these experiments and then tested before and after transformation by Rhim's in vitro methods (cell aggregation on soft agar and others). Subsequently, in vivo tumorigenicity in the nude mice was tested. Very good in vitro and in vivo correlations have been obtained in both chemical and viral transformation attempts.

V. Photochemical inactivation of cells and viruses

A novel inactivation method employing psoralens, a group of furocoumarins which react with nucleic acid when activated by long wave ultraviolet light, is being investigated. The experiments are in collaboration with Dr. Carl Hanson, a member of Dr. Lennett's professional staff and a biophysicist specializing in nucleic acids. He has close ties with the University of California Department of Chemistry and has high priority in obtaining their most promising newly synthesized psoralens. The psoralens are especially interesting as inactivating agents because:

A. They have the ability to freely permeate intact living cells and viruses, reacting with the nucleic acid strain in situ upon activation by long wave UV light.

B. They are reactive with nucleic acids only, and thus leave the most antigenic components of cells and viruses, i.e., proteins, glycoproteins, lipids, etc., intact.

C. The reaction with nucleic acid when properly carried out results in extensive adducts and crosslinks in the nucleic acid, thus completely blocking replication and transcription of the genome with the higher drug dosages. These alterations are so frequent along the DNA or RNA molecule that repair could not take place.

D. At least two of the psoralens have already had widespread human application in the treatment of psoriasis and other dermatologic problems, including recently a malignancy, mycosis fungoides. They have so far proven completely non-toxic for man at levels beyond their in vitro efficacy.

Preliminary experiments have shown that we can inactivate tumor cells with low doses of drugs; even the lowest dose tested, 0.02 mcg/cc, which leaves some cellular cytoplasmic mechanisms including attachment to surface, elongation and respiration intact for up to 2 weeks, completely stops all replication. Higher doses stop all vital processes.

Type C sarcoma viruses have been inactivated intracellularly with a new derivative, 4' aminomethyl trioxsalen, which is especially suitable for viruses in general; other DNA and RNA virus prototypes have been shown sensitive in independent experiments. The inactivated viruses seem to preserve physical and chemical integrity.

Killed cellular vaccines prepared from psoralenized tumor cultures seem more protective than formalin killed cultures, but have not yet been adequately quantitated.

Significance to Biomedical Research and the Program of the Institute:

Biology of tumorigenesis and tumor rejection in well-characterized mouse strains is efficient and economical, conserving funds as well as time-effort, provided the experiments are designed to be applicable to the human cancer problems. The mice we selected for experimentation are those which are most comparable to man in their natural tumor incidence, virus expression and immune response. In those mice it can be determined which type of vaccine offers the greatest promise for immunoprevention and immunotherapy of cancer, so that fewer costly primate trials are needed.

Transplantation of human and other animal tumors to immunodeficient mice is proving to be a useful tool in the study of cell malignancy and transformation.

Proposed Course:

Continuation of all segments described.

Publications:

Arnstein, P., Riggs, J.L., Oshiro, L.S., Huebner, R.J., and Lennette, E.H.: Induction of lymphoma and associated xenotropic type C virus in C57L mice by whole-body irradiation. J. Natl. Cancer Inst. 57: 1085-1090, 1976.

Neubauer, R.H., Rabin, H., Arnstein, P., Woodside, N., Valerio, M.G., Wallen, W.C., Hopkins, R.F.,III, and Loeb, W.F.: Characterization of a spontaneous undifferentiated carcinoma from an African green monkey (Cercopithecus aethiops). In Vitro 12: 533-539, 1976.

Rasheed, S., Gardner, M.B., Rongey, R.W., Nelson-Rees, W.A. and Arnstein, P.: Human bladder carcinoma: characterization of two new tumor cell lines. J. Natl. Cancer Inst., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04940-10-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Viruses in Experimental Oncogenesis and Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.A. Aaronson	Asst. Chief, Lab RNA Tumor Viruses	LRTV	NCI
Other:	S.R. Tronick	Microbiologist	LRTV	NCI
	M. Barbacid	NIH Visiting Fellow	LRTV	NCI
	S. Hino	Expert	LRTV	NCI
	C.D. Cabradilla	Chemist	LRTV	NCI
	K.J. Porzig	Staff Associate	LRTV	NCI
	J.M. Krakower	Staff Associate	LRTV	NCI

COOPERATING UNITS (if any)

R.J. Huebner, LRTV	P. Sarma, LRTV	R. Wilsnack, Huntington Lab
G. Kelloff, LRTV	B. Hampar, LDTV	M. Essex, Harvard Univ.
J.R. Stephenson, FCRC	K.C. Robbins, HLA	M. Phillips. Univ. of Pennsylvania
	R.V. Gilden, FCRC	L. Crittenden, U.S. Dept. Agric.

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	1.0	4.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are to elucidate the mechanisms of action of tumor viruses and to search for evidence of tumor viruses in humans with the eventual aim of developing rational approaches to prevention of human cancer. Topics of present interest include 1) the biology of endogenous type-C RNA viruses, 2) the molecular biology of type-C virus replication and RNA tumor virus transformation, and 3) the application of knowledge gained from these studies to the search for tumor viruses of humans.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To study the mechanisms of action of RNA and DNA containing tumor viruses.

2. To apply knowledge gained from experimental systems to search for a viral etiology to human neoplasia and to develop rational approaches to prevention and treatment of human tumors.

Methods Employed:

Standard and developmental techniques in virology, cell biology, immunology, and biochemistry.

Major Findings:

1. Type-C RNA viruses have been shown to exist as endogenous viruses within the cellular genomes of many mammalian species. A major research effort is aimed at devising immunological approaches for detection of endogenous virus expression in human tissues. Techniques have been successfully developed for the detection and partial purification of several type-C virus-coded proteins from virus-negative cells of a variety of species. During the past year, efforts have focused on the search for viral antigens in primate tissues. It has been possible to show that species representing each of the major genera of Old World primates express antigens related to the major structural proteins of the baboon-RD114 virus group. These antigens were shown to have diverged sufficiently so as to be immunologically distinguishable. Differences in the levels of expression of endogenous virus-coded proteins in tissues of a variety of primate species suggest differences in cellular regulation of virus expression by these species. With highly sensitive immunological techniques now available, analogous approaches are being utilized in the search for evidence of endogenous virus expression in higher apes and man.

2. A major area of investigation concerns the role of endogenous viruses in cancer. Previous studies have shown that endogenous viruses of mouse strains, characterized by high incidences of leukemia, replicate well in cells of those strains. Virus inoculation of susceptible mice that lack these viruses leads to the development of leukemia. These findings establish that inducible endogenous viruses can be oncogenic. Huebner and coworkers have previously shown that a B-tropic leukemia virus, isolated from old-aged BALB/c mice is oncogenic in the BALB/c mouse. This virus cannot be directly induced from BALB/c cells in tissue culture. However, by molecular hybridization and radioimmunologic analysis, the B-tropic virus was shown to be very closely related to a known N-tropic endogenous virus of the BALB/c strain. Moreover, genetic information of the B-tropic leukemia virus was demonstrated to segregate with a single structural locus conferring inducibility to the N-tropic endogenous virus.

The evidence is consistent with the hypothesis that the B-tropic virus arises through a minor genetic alteration that results in conversion of the N-tropic viruses to B-tropism. These findings demonstrate the importance of genetic controls over endogenous virus expression, since the lack of Fv-1 cellular restriction to B-tropic virus replication in the BALB mouse is associated with the development of neoplasia.

3. A viral etiology to cancer would be strongly implied by prevention of tumors through immunological approaches directed against virus infection and/or endogenous virus expression. Recent studies, performed in collaboration with Drs. Kelloff and Stephenson, LRTV, have led to the demonstration that the incidence of radiation-induced leukemia in the C57/B16 strain can be significantly reduced by passive or active immunization against the mouse type-C virus. These findings, along with those of Huebner and coworkers in other systems, help to demonstrate that the endogenous viruses are etiologically involved in cancers. They indicate further that immunoprevention may be a feasible and worthwhile approach toward prevention of tumors caused by endogenous viruses.

4. Investigations of cellular processes that regulate endogenous virus expression have the potential of providing information concerning how the cell represses potentially malignant viral genes. Previous studies in the mouse have defined at least three classes of endogenous type-C virus, each of which appears to be subject to specific regulatory factors of the host cell. One virus, known to be present in a large number of inbred strains, has been shown to be inducible by inhibitors of protein synthesis under conditions where other viruses present in the same cell are not activated. Treatment of BALB/c mouse cells with a variety of inhibitors or protein synthesis results in a marked increase in the level of virus-specific RNA and subsequent virus release. It has been possible to demonstrate that the increase in virus-specific RNA is due to an increase in the rate of RNA synthesis rather than prevention of its degradation. These findings indicate that expression of this class of endogenous virus is normally inhibited by a labile repressor-like protein that acts at a transcriptional level.

5. Another group of agents that derepress cellular control over endogenous viruses includes naturally occurring antigens such as lipopolysaccride (LPS), a component of certain bacteria. LPS and analogous components of other bacterial strains have been shown to specifically and efficiently induce xenotropic endogenous viruses from lymphoid cells of a number of inbred strains. The primary target of these inducing agents has been shown to be the B-lymphoid cell. T cells, as well as embryo fibroblasts, are not virus-inducible by these same bacterial antigens. Studies are in progress to define the functional significance of such naturally occurring virus inducers of specific lymphoid cell populations.

6. A new group of vertebrates has been shown to contain endogenous viruses. It has been possible to induce type-C virus from cells of a New World mammal, Columbian black-tailed deer (*O. hemionus*). Viral cDNA prepared from this endogenous virus has been shown to detect related genetic

sequences in a wide variety of ungulates, including those of both New and Old World origin. The evidence indicates that endogenous viruses have been genetically transmitted in these mammals for several million years and have existed in the New World for a period of at least five to ten million years.

7. The development of immunologic approaches for study of different proteins of mammalian type-C viruses has led to the establishment of immunoassays for each of the known viral-coded proteins. These included p30, p15, p12, and p10, each of which is coded as part of a polyprotein precursor of the gene, designated gag. The viral reverse transcriptase(pol gene) and the envelope glycoprotein, gp70 (env gene) have all been characterized physically and by radioimmunoassay. The virus-coded nature of each protein as well as their unique antigenic determinants have been established. In the broadest interspecies immunoassays for each of these viral proteins, it has been possible to demonstrate that interspecies antigenic determinants are shared between the analogous p30s, p10s, p15s, and reverse transcriptases, respectively, of all known mammalian type-C viruses. These findings strongly argue for a common progenitor in the evolution of mammalian type-C viruses.

8. Type-C viruses of several species have been demonstrated to be oncogenic in their natural hosts. In contrast to endogenous viruses, known oncogenic type-C viruses are generally horizontally transmitted as infectious agents. Exposure of the animal to such horizontally transmitted viruses generally leads to the development of an immune response directed against viral structural proteins. In the cow, for example, it has been possible to show that antibody to bovine lymphosarcoma virus is present in essentially all tumored animals. In contrast, no detectable antibody to this virus was detected in animals of a low leukemia incidence herd. Of note, in random samples of herds in both the U.S. and Canada, as many as 5 to 20% of animals have antibody to this virus. These findings suggest that the prevalence of bovine leukemia virus in cattle is much higher than has been suspected on the basis of clinical findings. In other studies, cats and gibbon apes have been shown to contain high-titered antibodies to horizontally transmitted oncogenic type-C viruses. To date, human sera have not shown evidence of exposure to these viruses by analogous immunological assays. Included have been sera from over 200 laboratory workers involved in viral oncology research. The fact that reactivity to type-C viral proteins has not been detected in sera of normal or cancer patients tested argues that classical horizontal infection with known mammalian type-C viruses must be rare. Efforts to detect serologic reactivity in humans against proteins of RNA tumor viruses are being continued, especially as protein probes are obtained from new virus isolates.

9. Recently, several type-C viruses have been isolated from cultured human cells by workers in other laboratories. These viruses have been characterized by means of radioimmunoassays for highly type-specific

type-C viral proteins. Such assays have proven extremely useful in virus identification. To date, several type-C virus isolates of putative human origin have been shown by these techniques to be indistinguishable immunologically from known laboratory viruses.

10. Essex, Hardy and coworkers have reported an antigen, designated FOCMA, exists on the cell surface of cat leukemias and sarcomas. Of note, cats with high-titered antibody to FOCMA appear to be resistant to horizontally transmitted FeLV-induced disease. The relationship of FOCMA to known FeLV structural proteins has not been previously clarified. In studies performed in collaboration with Dr. Essex, it was possible to demonstrate that the antibody to FOCMA was independent of antibody to either of the major FeLV structural proteins, p30 or gp70. These findings argue strongly against the possibility that FOCMA is either of these FeLV-coded structural proteins.

11. In studies designed to elucidate the structure of the type-C viral genome, the order of the gag gene of mammalian type-C viruses has been established. The presence of specific virus-coded proteins in different MW precursors in cells transformed by replication-defective transforming viruses and cells infected with a leukemia virus mutant, temperature-sensitive for cleavage of the gag gene precursor, has been analyzed. These findings made it possible to deduce the order of the murine viral gag gene as 5'-p15-p12-p20-p10-3'.

12. A reverse transcriptase-containing virus, designated Mason-Pfizer monkey virus (MPMV), was initially isolated from a breast tumor of a rhesus monkey. Subsequent isolates have also been obtained from normal rhesus monkeys. MPMV differs in both its structure and the antigenic determinants of its major structural protein from those of any known type-C virus. However, MPMV, like mammalian type-C viruses, contains a glycoprotein whose molecular weight is approximately 70,000. An interspecies immunoassay utilizing antiserum to MPMV to precipitate baboon viral gp70 has been shown to detect antigenic determinants shared by glycoproteins of MPMV, RD114, and baboon type-C viruses. These findings indicate that MPMV may have originated by recombination with endogenous primate type-C viral genetic sequences.

13. An important group of RNA tumor viruses consists of viruses that transform fibroblasts in tissue culture. To date, all mammalian transforming viruses have been shown to be defective in functions necessary for their replication. A large number of morphologic revertants of sarcoma virus-transformed nonproducer cells have been isolated and characterized. Two major classes of revertants of the same clonal transformant have been characterized. One expresses sarcoma viral RNA at a level comparable to that of the wild-type transformant. Retransformation of such revertants occurs spontaneously at a low frequency. Along with other evidence the results indicate that this class of revertants contains transforming viruses that are reversibly altered as a result of point mutations of the integrated sarcoma viral genome. Attempts to detect complementation or

recombination between such mutant sarcoma viruses present in different clonal revertant lines have so far been unsuccessful. Another class of revertants has been shown to lack detectable sarcoma viral information and thus, appears to have arisen through loss of the integrated transforming virus. Further studies to elucidate both cellular and viral functions required for expression of the sarcoma virus should lead to better understanding of the virus-transformed state.

14. Continued efforts to isolate and characterize cell lines established from cultured human tumors have led to an increasing number of permanent cell lines derived from carcinomas, sarcoma and melanomas. Investigations of mechanisms responsible for malignant change in human tumor cells are being performed. Efforts are also in progress to establish normal primate cell culture systems that can be utilized for in vitro transformation by chemicals as well as by viruses in combination with chemicals. These studies are being pursued to investigate how chemical carcinogens, acting alone or in concert with viruses, cause neoplastic transformation.

Significance to Biomedical Research and the Program of the Institute:

The systems that are being intensively investigated have provided a much better understanding of the biology and biochemistry of viral transformation. It is felt that a clear understanding of these phenomena will significantly speed progress in the search for a viral etiology of human cancer as well as approaches toward cancer prevention.

Proposed Course:

To continue research already in progress in the following major areas: (1) mechanisms of action of murine sarcoma and murine leukemia viruses; (2) induction and regulation of type-C viruses in virus-negative cells; (3) determination of the role of viruses in human neoplasia; and (4) to develop new research areas in model systems that pertain to human disease.

Publications:

Aaronson, S.A. and Stephenson, J.R.: Viruses and the etiology of cancer. In Drew, W.L. (Ed.): Clinical Concepts in Virology. Philadelphia, F.A. Davis, 1976, pp. 251-292.

Aaronson, S.A. and Schlom, J.: The search for RNA tumor viruses in human cancer. In Ariel, I. (Ed.): Progress in Clinical Center. New York, Grune & Stratton, Inc., 1976, pp. 51-63.

Kelloff, G.J., Peters, R.L., Donahoe, R.M., Stephenson, J.R. and Aaronson, S.A.: Natural occurrence of tumors in mouse strains differing in their xenotropic and ecotropic endogenous viruses. J. Nat. Cancer Inst. 57: 85-89, 1976.

Phillips, S.M., Stephenson, J.R., Greenberger, J.S., Lane, P.E. and Aaronson, S.A.: Release of xenotropic type-C RNA virus in response to lipopolysaccharide: activity of lipid-A portion upon "B" lymphocytes. J. Immun. 116: 1123-1128, 1976.

Smith, E.J., Stephenson, J.R., Crittenden, L.B. and Aaronson, S.A.: Avian leukosis-sarcoma virus gene expression: noncoordinate control of group specific antigens in virus-negative avian cells. Virology 70: 493-501, 1976.

Aaronson, S.A. and Stephenson, J.R.: Intracellular and systemic regulation of biologically distinguishable type C RNA viruses of mouse cells. In Hanna, M.G. and Rapp, F. (Eds.): Contemporary Topics in Immunobiology. New York, Plenum Publishing Corp., 1977, pp. 107-126.

Stephenson, J.R. and Aaronson, S.A.: Induction of an endogenous B-tropic type-C RNA virus from SWR/J mouse embryo cells in tissue culture. Virology 70: 352-359, 1976.

Aaronson, S.A., Stephenson, J.R., Tronick, S.R. and Hino, S.: Immunological analysis of structural polypeptides of woolly monkey-gibbon ape type C viruses: woolly monkey sarcoma virus. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research, Bibl. Haemat., 43. Basel, Karger, 1976, pp. 102-109.

Okabe, H., Gilden, R.V., Hatanaka, M., Stephenson, J.R., Gallagher, R.E., Gallo, R.C. and Aaronson, S.A.: Immunologic and biochemical characterization of type-C viruses isolated from cultured human AML cells. Nature 260: 264-266, 1976.

Hampar, B., Aaronson, S.A., Derge, J.G., Chakrabarty, M., Showalter, S.D. and Dunn, C.Y.: Activation of an endogenous mouse type-C virus by UV-irradiated herpes simplex virus types 1 and 2. Proc. Nat. Acad. Sci. U.S.A. 73, 646-650, 1976.

Greenberger, J.S., Bensinger, W.I. and Aaronson, S.A.: Selective techniques for the isolation of morphologic revertants of sarcoma virus transformed cells. In Prescott, D. (Ed.): Methods in Cell Biology. New York,Academic Press, 1976, pp. 237-248.

Aaronson, S.A., Stephenson, J.R., Hino, S. and Cabradilla, C.: Endogenous type C RNA viruses of mouse cells: a model for the study of gene regulation in eukaryotes. In Criss, W.E., Ono, T. and Sabine, J.R. (Eds.): Proc. Seventh Int. Symp. on Modified Cellular and Molecular Controls in Neoplasia. New York, Raven Press, 1976, pp. 279-294.

Stephenson, J.R. and Aaronson, S.A.: Search for antigens and antibodies cross-reactive with type-C viruses of the woolly monkey and gibbon ape in animal models and in humans. Proc. Nat. Acad. Sci. U.S.A. 73: 1725-1729, 1976.

Aaronson, S.A. and Stephenson, J.R.: Endogenous type-C RNA viruses of mammalian cells. Biochimica et Biophysica Acta 458: 323-354, 1976.

Stephenson, J.R., Cabradilla, C.D. and Aaronson, S.A.: Genetic factors influencing endogenous type-C RNA viruses of mouse cells: control of viral polypeptide expression in the C57BL/10 strain. Intervirology 6: 258-269, 1976.

Hino, S., Stephenson, J.R. and Aaronson, S.A.: Radioimmunoassays for the 70,000 molecular weight glycoproteins of endogenous mouse type-C viruses: viral antigen expression in normal mouse tissues. J. Virol. 18: 933-941, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Structural polypeptides of mammalian type-C RNA viruses: isolation and immunologic characterization of a low molecular weight polypeptide, p 10. J. Biol. Chem. 251: 4859-4866, 1976.

Stephenson, J.R., Hino, S., Garrett, E.W. and Aaronson, S.A.: Immunological cross-reactivity of Mason-Pfizer monkey virus with type-C RNA viruses endogenous to primates. Nature 261: 609-611, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: The gag gene of mammalian type-C RNA tumor viruses. Nature 262: 554-559, 1976.

Aaronson, S.A., Tronick, S.R. and Stephenson, J.R.: Type-C RNA virus isolation from the black-tailed deer, Odocoileus hemionus: evidence for endogenous viruses of New World origin. Cell 9: 489-494, 1976.

Stephenson, J.R., Hino, S., Peters, R.L., Donahoe, R.M., Long, L.K., Aaronson, S.A. and Kelloff, G.J.: Natural immunity in mice to structural polypeptides of endogenous type-C RNA viruses. J. Virol. 19: 890-898, 1976.

Cabradilla, C.D., Robbins, K.C. and Aaronson, S.A.: Mouse type-C virus induction by translational inhibitors: evidence for transcriptional derepression of a specific class of endogenous virus. Proc. Nat. Acad. Sci. U.S.A., 73: 4541-4545, 1976.

Devare, S.G., Stephenson, J.R., Chander, S., Sarma, P.S. and Aaronson, S.A.: Bovine lymphosarcoma: development of a sensitive radioimmunologic technique for detection of the etiologic agent. Science 194: 1428-1430, 1976.

Essex, M., Cotter, S.M., Sliski, A.H., Hardy, W.D., Jr., Stephenson, J.R., Aaronson, S.A. and Jarrett, O.: Horizontal transmission of feline leukemia virus under natural conditions in a feline leukemia cluster household. Int. J. Cancer, 19: 90-96, 1977.

Phillips, S.M., Stephenson, J.R. and Aaronson, S.A.: Genetic factors influencing mouse type-C RNA virus induction by naturally occurring B-cell mitogens. J. Immunol., 118: 662-666, 1977.

Stephenson, J.R., Barbacid, M., Tronick, S.R., Hino, S. and Aaronson, S.A.: Proteins of type-C RNA tumor viruses. Recent Advances in Cancer Research. In press.

Stephenson, J.R. and Aaronson, S.A.: Differential regulation of endogenous type-C viruses of mouse cells. Microbiology. In press.

Bensinger, W.I., Robbins, K.C., Greenberger, J.S. and Aaronson, S.A.: Different mechanisms for morphologic reversion of a clonal population of murine sarcoma virus-transformed nonproducer cells. Virology. In press.

Stephenson, J.R., Essex, M., Hino, S., Hardy, W.D., Jr. and Aaronson, S.A.: Feline oncornavirus-associated cell membrane antigen (FOCMA). VII. Distinction between FOCMA and the major virion glycoprotein. Proc. Nat. Acad. Sci. U.S.A. In press.

Essex, M., Stephenson, J.R., Hardy, W.D., Jr., Cotter, S.M. and Aaronson, S.A.: Leukemia, lymphoma and fibrosarcoma of cats as models for similar diseases of man. In Hiatt, H.H., Watson, J.D. and Winston, J.A. (Eds.): Origins of Human Cancer. Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y. In press.

Stephenson, J.R. and Aaronson, S.A.: Endogenous type-C viral expression in primates. Nature. In press.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Evolutionary relationships between gag gene-coded proteins of murine and primate endogenous type-C RNA viruses. Cell. In press.

Peters, R.L., Sass, B., Stephenson, J.R., Al-Ghazzouli, I.K., Hino, S., Donahoe, R.M., Kende, M., Aaronson, S.A. and Kelloff, G.J.: Immunoprevention of x-ray induced luekemias in the C57BL mouse. Proc. Nat. Acad. Sci. U.S.A. In press.

Krakower, J.M., Barbacid, M. and Aaronson, S.A.: Radiommunoassay for mammalian type-C viral reverse transcriptase. J. Virol. In press.

Robbins, K.C., Cabradilla, C.D., Stephenson, J.R. and Aaronson, S.A.: Origin of a B-tropic leukemia virus of the BALB/c mouse strain. Proc. Nat. Acad. Sci. U.S.A. In press.

Tronick, S.R., Golub, M.M., Stephenson, J.R. and Aaronson, S.A.: Distribution and expression in mammals of genes related to an endogenous type-C RNA virus of Odocoileus hemionus. J. Virol. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04941-05-LRTV

PERIOD COVERED
July 1, 1976, to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical Studies of RNA tumor viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI	- Steven R. Tronick, Microbiologist	LRTV	NCI
OTHER	- S. A. Aaronson, Asst. Chief, Lab RNA Tumor Viruses	LRTV	NCI
	J. R. Stephenson, Head, Viral Genetics Section	LRTV	NCI
	S. Hino, Expert	LRTV	NCI
	M. Barbacid, NIH Visiting Fellow	LRTV	NCI
	C. D. Cabradilla, Chemist	LRTV	NCI
	J. M. Krakower, Research Associate	LRTV	NCI
	K. Porzig, Research Associate	LRTV	NCI

COOPERATING UNITS (if any)
R. J. Huebner, LRTV; R. E. Wilsnack, Huntington Lab.; W. A. Haseltine, Harvard Med. Sch.
J. Dahlberg, LRTV; I. M. Verma, Salk Institute
K. Robbins, Hazleton Labs; P. Anderson, Wistar Institute

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to biochemically and immunologically characterize type C RNA viruses in order to understand the mechanisms by which these viruses transform cells. Another goal of this research is to determine whether or not these viruses play a role in the etiology of human cancers. Studies currently in progress are the following: 1) Biochemical characterization of new isolates of type C viruses; 2) Studies of the cellular control of endogenous viral gene expression in normal and malignant cells: 3) Search for the presence of type C viral genes and gene products in human tumors; 4) Biochemical characterization of mammalian transforming viruses.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To biochemically characterize new isolates of reverse transcriptase-containing viruses from mammalian species.

2. To study the cellular regulation of the expression of the type C viral genome by using molecular hybridization techniques.

3. To determine the involvement of reverse transcriptase-containing viruses in cancers of primates and other mammalian species.

Methods Employed:

Standard biochemical techniques for nucleic acid isolation and analysis; assays for enzymes of nucleic acid synthesis and degradation: radioimmunoassays for qualitative and quantitative characterization of tumor viruses and their proteins; molecular hybridization techniques using single-strand specific nuclease and hydroxyapatite chromatography to detect and characterize viral genomes.

Major Findings:

1. A reverse transcriptase-containing virus was isolated in our laboratory by cocultivation of human and black-tailed deer cells. Cocultivation of the same deer cell line with horse cells also led to virus production. The viral reverse transcriptase was shown to possess catalytic and antigenic properties in common with known mammalian type C viral enzymes. An antiserum to disrupted virus was prepared and was tested for its ability to bind ^{125}I-p30 proteins isolated from a number of type C viruses. The antiserum bound RDl14 and baboon viral p30s, and also p30s from MuLV, FeLV, and woolly monkey type C virus, but to much lower extents. Binding to AMV ^{125}I-p30 and MPMV ^{125}I-p26 could not be detected. A competition radio-immunoassay for the antigen of the newly isolated virus was developed using the antiserum to bind ^{125}I-RD p30. In this assay the new virus, RDl14, and baboon type C viruses gave complete cross-reactions, whereas, all other known type C viruses reacted to much lower degrees. The new virus isolate could be readily distinguished from RDl14 and baboon type C viruses in an homologous immunoassay for RDl14 p30. A highly radioactive cDNA was synthesized using the endogenous reverse transcriptase activity of the new virus isolate. The cDNA was used to establish the genetic relatedness of the new isolate to known RNA tumor viruses. No detectable hybridization was observed between this cDNA and the RNAs of representatives of the major groups of mammalian type C viruses, although the cDNA hybridized extensively to its homologous viral RNA. To establish the origin of the new virus, the cDNA was used in hybridization reactions with DNA isolated from human, deer, and horse tissues. Only DNA from deer (Odocoileus hemionus) hybridized, thus demonstrating the origin of the virus and also establishing it as an endogenous type C virus of the black-tailed deer. The O. hemionus type C

virus is the first to be isolated from a mammalian species of New World origin. Using molecular hybridization techniques, the distribution in mammals of nucleotide sequences homologous to the O. hemionus viral genome was determined. The greatest extents of hybridization were observed with DNAs isolated from Odocoileus species (85% hybridization). DNA from deer species of other genera (family Cervidae) hybridized to the extent of 40%. These latter species all are of Old World origin. Hybridization (20%) was also detected with DNA of members of a more distantly related artiodactyl family (Bovidae). This is the first demonstration of endogenous type C viral genes in bovid species. DNA isolated from members of the artiodactyl families Camelidae and Suidae, did not hybridize with the deer viral cDNA. These families diverged from cervids about 55 million years ago in contrast to 30 million years for the divergence of cervids and bovids. DNAs isolated from primate, rodent, feline, canine, and equine species also did not react. The partial genetic homology observed between the cDNA and DNA of Old World cervid species and bovids was shown by Tm analysis to be due to random base mismatches rather than to large scale deletions or additions of viral related sequences. The rate of divergence of type C viral sequences in cervids and bovids appeared to be somewhat faster than the rate of sequence divergence of unique sequence cellular DNAs of these same species. The expression of endogenous type C viral genes in cervids was also studied using the radioimmunoassay for O. hemionus viral antigen. Normal tissues of the white-tailed deer and caribou contained a cross-reactive antigen that had the same physical and biochemical properties of authentic type C viral p30. That type C viral genes have been preserved for at least 30 million years in artiodactyls and have maintained their ability to be expressed, suggests the existence of positive evolutionary selective pressure for the maintenance of type C viral genes.

Significance to Biomedical Research and the Program of the Institute:

The availability of highly sensitive and specific biochemical and immunological probes for type C viral gene products has become increasingly of value in order to both demonstrate the possible etiologic involvement of these viruses in human cancers and to study the role that type C viral gene products may play in normal cellular functions.

Proposed Course:

The distribution of O. hemionus type C viral genes in mammals will be studied in more detail in order to learn more about its origin. New type C virus isolates will also be studied in a similar manner. Molecular hybridization will be used to study the genetics and cellular regulation of type C viruses.

Publications:

Ablashi, D. V., Oie, H. K., Armstrong, G. R., Didier-Fichet, M. L., Tronick, S. R., Heine, V. and Dalton, A. J.: Xenotropic properties of an isolate from murine Rauscher leukemia virus. J. Med. Primatol. 5: 223-236, 1976.

Okabe, H., Gilden, R. V., Hatanaka, M., Stephenson, J. R., Tronick, S. R., Gallagher, R. E., Gallo, R. C. and Aaronson, S. A.: Immunologic and biochemical characterization of type C virus isolates from cultured AML cells. Nature 260: 264-266, 1976.

Tronick, S. R., Golub, M. M., Stephenson, J. R. and Aaronson, S. A.: Distribution and expression in mammals of genes related to an endogenous type C RNA virus of Odocoileus hemionus. J. Virol. (In press, 1977).

Aaronson, S. A., Tronick, S. R. and Stephenson, J. R.: Endogenous Type C RNA virus of Odocoileus hemionus, a mammalian species of New World origin. Cell 9: 489-494, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04942-07-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cellular Regulation of Endogenous Type C RNA Viruses of Mammalian Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. R. Stephenson	Head, Viral Genetics Section	LRTV NCI
OTHER:	S. G. Devare	NIH Visiting Fellow	LRTV NCI
	R. K. Reynolds	Microbiologist	LRTV NCI

COOPERATING UNITS (if any)

R.J. Huebner, LRTV,NCI	M. Phillips, U. Penn.	M. Essex, Harvard U.
S.A. Aaronson, LRTV,NCI	F. Reynolds, FCRC	R.V. Gilden, FCRC
G.J. Kelloff, LRTV, NCI	G.J. Todaro, LVC,NCI	M.B. Gardner, USC
	E. Fleissner, Sloan-Kettering	M. Barbacid, LRTV,NCI

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Viral Genetics Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major emphasis of this research involves an analysis of type C RNA virus expression in different mammalian species, investigation of cellular regulatory controls influencing expression of endogenous type C RNA viruses, and determination of the possible etiologic role of RNA type C viruses in natural tumors of their hosts. The eventual goals of these studies will be aimed towards the development of rational approaches to prevention of human cancer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To study the mechanisms of replication and transformation by type C RNA viruses, investigate cellular regulatory controls influencing expression of endogenous type C viruses and determine the role of these viruses in human neoplasia.

Methods Employed:

1. Biochemical and immunologic techniques for isolation and analysis of viral structural proteins: radioimmunoassays for proteins of different classes of mammalian type C RNA viruses.

2. Standard tissue culture procedures, including XC plaque assays for leukemia virus and focus forming assays for sarcoma virus.

Major Findings:

1. Endogenous type C RNA tumor virus genetic sequences are known to be naturally integrated within the host cell genome of many vertebrate species. The isolation of endogenous type C viruses of primates has been limited to species of the genus Papio. Expression of a protein immunologically cross-reactive with the major structural protein of a type C virus isolate of the Papio cynocephalus baboon was demonstrated in tissues of each of fourteen species, representing five major genera of Old World monkeys. In contrast, using a similar approach, no evidence of type C viral antigenic reactivity was detected in over 100 normal and tumor tissues of higher apes or humans. Immunologic analysis of viral antigen partially purified from Old World monkey tissues indicated significant antigenic difference between individual genera. Differential regulation of endogenous virus expression even by different genera of primates was indicated by the high levels of viral antigen in tissues of species of the genus Macaca as compared to the genus Papio, despite the high frequency of virus isolation from tissues of the genus Papio. The inability to detect similar antigens in tissues of apes or man may reflect differences in regulation. Alternately, antigens coded by the endogenous viral genomes of apes and man may have diverged to the extent that they are no longer reactive by currently available assay procedures.

2. Several low molecular weight proteins of endogenous viruses of the RD114/baboon group were compared with the gag gene-coded translational products of endogenous type C viruses of murine origin. The p10 proteins of each virus group were shown to be immunologically and biochemically related, while the p12 proteins of RD114/baboon viruses were demonstrated to share antigenic determinants with murine viral p15. Moreover, highly-type specific phosphoproteins, p15 of RD114/baboon viruses and p12 of murine viruses, were shown to possess very similar biochemical properties. These findings,

along with previous studies indicating immunologic cross-reactivity between their major internal antigens, p30, demonstrate that each of the gag gene-coded proteins of murine type C viruses has an analogue in viruses of the RD/114 baboon group. The immunologic and biochemical relatedness of their gag gene translational products supports the concept of a common progenitor in the evolution of these endogenous viruses.

3. The genome of mammalian type C RNA viruses includes sequences coding for an envelope glycoprotein, env gene, the viral reverse transcriptase, pol gene, and a gene designated gag which codes for several viral structural proteins. Virion proteins coded for within the gag gene are synthesized in the form of a 65,000 molecular weight precursor polypeptide which becomes subsequently cleaved to proteins with molecular weights of 30,000 (p30), 15,000 (p15), 12,000 (p12) and 10,000 (p10). An analysis of viral antigen expression in cells infected with MuLV mutants defective in precursor cleavage and cells nonproductively transformed by various sarcoma virus isolates led to the identification of several intermediate cleavage products. By this means the gag gene sequence was deduced to be 5'-p15-p12-p30-p10-3'.

4. An endogenous type C virus was isolated from the Columbian black-tailed deer (Odocoileus hemionus). While this virus shares biochemical and immunologic characteristics with other mammalian type C viruses, its immunologic properties readily distinguish it from known endogenous viruses. By DNA-DNA hybridization, extensive homology was demonstrated between the viral cDNA and cellular DNA isolated from Odocoileus species. DNAs of representatives of other genera within the same family, Cervidae, were partially related to the virus, consistent with the phylogenetic relationship of these species to Odocoileus. O. hemionus viral sequences were also detected within cellular DNAs of members of a more distantly related artiodactyl family, Bovidae. These findings suggest the genetic transmission of type C viral genes within cervids and bovids for a period of at least 25-30 million years. By radioimmunologic techniques, it was possible to detect and partially purify, from normal tissues of cervid species, antigens related to the major structural protein of the O. hemionus virus. The present findings that O. hemionus virus has been genetically transmitted for millions of years, and yet has maintained the ability to be expressed as infectious virus, argue for positive evolutionary selective pressures for the maintenance of type C viral genes.

5. A B-tropic type C RNA virus isolatable from lymphoreticular tumors of the inbred BALB/c mouse strain has previously been shown to be leukemogenic in its natural host. This virus is not chemically inducible from BALB/c embryo cells or from embryo lines containing segregating inducibility loci for two known endogenous type C viruses of BALB/c cells. Molecular hybridization and type-specific immunologic assays demonstrated a very high degree of genetic homology between the B-tropic leukemia virus and BALB:virus-1, an N-tropic endogenous virus of BALB/c cells. Genetic sequences specific for BALB:virus-1 were shown to segregate with the locus for BALB:virus-1

induction in genetic crosses between BALB/c and the noninducible NIH Swiss strain. Thus, information for the B-tropic virus appears to be closely linked to the structural locus for BALB:virus-1 within the BALB/c genome. The evidence is consistent with a mechanism by which a small genetic alteration affecting a normally well-controlled endogenous virus leads to virus expression and the subsequent development of neoplasia.

6. The expression of endogenous type C viral proteins in avian embryo cells was examined. Competition immunoassays were developed for the proteins coded by the gag, pol and env genes. Avian embryo cells productively infected with an endogenous avian type C virus, RAV-0, were found to express gag and env gene coded proteins at coordinate levels while the levels of RNA-dependent DNA-polymerase were at least two orders of magnitude lower. A similar pattern of viral antigen expression was found in virus-negative avian cells with the exception that the amino terminal protein of the gag gene product, p15, was not detectable. In addition, molecular sizing experiments revealed that cleavage of the gag gene-coded precursor protein was incomplete in these latter cells. These findings demonstrate two possible explanations for the restriction to virus production in these cells. These include the absence of p15 and incomplete proteolytic cleavage of the gag gene-coded precursor. Further, the noncoordinate expression of polymerase relative to the gag- and env-coded translation products suggests the existence of multiple initiation sites for protein synthesis within the avian RNA tumor virus genome.

7. A radioimmunoassay has been developed for antibody directed against the major 24,000 molecular weight (p24) protein of bovine leukemia virus (BLV). The binding of ^{125}I-labeled BLV p24 with sera produced by immunization with BLV but not by sera to known type C or type B viruses or other infectious viruses of bovine origin indicates the specificity of this assay. Moreover, immunoprecipitation of ^{125}I-labeled BLV p24 could be competed with extracts of BLV-infected fetal lamb cells but not uninfected fetal lamb or bovine cell extracts. Sera from 100% of adult cattle tested with clinically diagnosed lymphosarcoma exhibited high titered antibody against BLV p24. In contrast, sera from animals with the less common calf form of the disease lacked detectable antibody to BLV p24. Analysis of around 6000 sera from randomly selected dairy cattle from 21 States of America indicated the presence of antibody against BLV p24 in a high fraction of animals tested. The percentage of animals with detectable antibody to BLV ranged from a low of 2-4% to as high as 42%. Moreover, sera from a significant number of antibody-negative animals were shown by competition immunoassay to contain high levels of viral antigen. These findings indicate that the infection by this virus may be much more prevalent among domestic cattle than previously believed. Human sera from 450 individuals employed in slaughterhouses or on dairy farms as well as over 900 sera from patients with various forms of cancer failed to demonstrate detectable antibody to BLV p24.

8. The immunological responses of inbred mice to structural components of one class of endogenous virus were investigated by means of radioimmunoassays

utilizing highly purified viral proteins. Naturally occurring antiviral antibodies were demonstrated only in those strains possessing information for induction of a mouse cell-tropic endogenous virus. Moreover, these antibodies invariably appeared subsequent to the detection of spontaneous replication of this virus in the same animal. The immune responses elicited were much stronger against endogenous viral gp70 than p30, consistent with previous findings of tolerance in the mouse to the major structural antigen of its endogenous virus. However, the demonstration of an immune response to p30 under conditions of both natural and experimental immunization establishes that tolerance to this viral antigen can be overcome.

9. The 10,000 molecular weight gag gene-coded mammalian type C virus structural protein, p10, was shown to be virus-coded and to possess strong group-specific antigenic determinants. By use of appropriate immunoassays, broadly reactive interspecies determinants shared by mammalian type C virus isolates of murine, feline, and primate origin, were also demonstrated. The interspecies antigenic determinants of p10 were shown to be as broadly cross-reactive as those exhibited by the major type C virus structural polypeptide, p30.

10. The humoral antibody response of feline leukemia virus (FeLV) exposed cats to the feline oncornavirus associated tumor cell membrane antigen (FOCMA) is directly correlated with immunosurveillance against tumor development under natural conditions. Using membrane immunofluorescence and radioimmunoprecipitation, the antibody response to FOCMA was found to be independent from the antibody response to the major envelope and core proteins of FeLV, gp70 and p30. This was especially true for healthy viremic cats, where antigenemia with circulating FeLV gp70 and p30 apparently ties up any free antibody to these proteins, but high levels of FOCMA antibody are often concurrently present. Exhaustive in vitro absorption of highly immune non-viremic serum with gp70 and p30 also failed to remove FOCMA antibody activity. These results indicate that FOCMA is not one of the major FeLV structural proteins.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the genetic regulatory factors influencing expression of endogenous viruses of mouse cells should provide methods for analysis of type C viruses of primates and be of value in determining the role of such viruses in the etiology of natural tumors. The availability of highly sensitive and specific immunoassays for viral structural proteins will be of value in the analysis of primate tissues for virus expression and for the characterization of new type C virus isolates of potential human origin.

Proposed Course:

To further examine cellular regulatory controls influencing endogenous type C virus expression of mammalian cells, including those of primate origin, and to study the involvement of type C RNA viruses in natural tumors of different mammalian species.

Publications:

Stephenson, J.R. and Aaronson, S.A.: Induction of an endogenous B-tropic type C RNA virus from SWR/J mouse embryo cells in tissue culture. Virology 70: 352-359, 1976.

Okabe, H., Gilden, R.V., Hatanaka, M., Stephenson, J.R., Gallagher, R.E., Gallo, R.C. and Aaronson, S.A.: Immunologic and biochemical characterization of type C virus isolates from cultured human AML cells. Nature 260: 264-266, 1976.

Hino, S., Stephenson, J.R. and Aaronson, S.A.: Radioimmunoassays for the 70,000 molecular weight glycoproteins of endogenous mouse type C viruses: viral antigen expression in normal mouse tissues. J. Virol. 18: 933-941, 1976.

Aaronson, S.A., Stephenson, J.R., Tronick, S.R. and Hino, S.: Immunological analysis of structural polypeptides of woolly monkey-gibbon ape type C viruses: woolly monkey sarcoma virus. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research , Bibl. Haemat., 43. Basel, Karger, 1976, pp. 102-109.

Aaronson, S.A. and Stephenson, J.R.: Endogenous type C RNA viruses of mammalian cells. BBA Reviews on Cancer 458: 323-354, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Structural polypeptides of mammalian type C RNA viruses: isolation and immunologic characterization of a low molecular weight polypeptide, p10. J. Biol. Chem. 251: 4859-4866, 1976.

Stephenson, J.R., Hino, S., Garrett, E.W. and Aaronson, S.A.: Immunologic cross-reactivity of Mason-Pfizer monkey virus with type C RNA viruses endogenous to primates. Nature 261: 609-611, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: The gag gene of mammalian type C RNA tumor viruses. Nature 262: 554-559, 1976.

Aaronson, S.A., Tronick, S.R. and Stephenson, J.R.: Type C RNA virus isolation from the black-tailed deer, Odocoileus hemionus: evidence for endogenous viruses of New World origin. Cell 9: 484-494, 1976.

Stephenson, J.R., Cabradilla, C.D. and Aaronson, S.A.: Genetic factors influencing endogenous type C RNA viruses of mouse cells: control of viral polypeptide expression in the C57BL/10 strain. Intervirology 6: 258-269, 1976.

Smith, E.J., Stephenson, J.R., Crittenden, L.B. and Aaronson, S.A.: Avian leukosis-sarcoma virus gene expression: noncoordinate control of group-specific antigens in virus-negative avian cells. Virology 70: 493-501, 1976.

Stephenson, J.R., Hino, S., Peters, R.L., Donahoe, R.M., Long, L.K., Aaronson, S.A., and Kelloff, G.J.: Natural immunity in mice to structural polypeptides of endogenous type C RNA viruses. J. Virol. 19: 890-898, 1976.

Yeger, H., Kalnins, V.I. and Stephenson, J.R.: Electron microscopy of mammalian type C RNA viruses: use of conditional lethal mutants in studies of virion maturation and assembly. Virology 74: 459-469, 1976.

Devare, S.T., Stephenson, J.R., Chander, S., Sarma, P.S. and Aaronson, S.A.: Bovine lymphosarcoma: development of a sensitive radioimmunologic technique for detection of the etiologic agent. Science 194: 1428-1430, 1976.

Phillips, S.M., Stephenson, J.R. and Aaronson, S.A.: Genetic factors influencing mouse type C RNA virus induction by naturally occurring B-cell mitogens. J. Immunol. 118: 662-666, 1977.

Aaronson, S.A., Stephenson, J.R., Hino, S. and Cabradilla, C.: Endogenous type C RNA viruses of mouse cells: a model for the study of gene regulation in eukaryotes. In Criss, W.E., Ono, T. and Sabine, J.R. (Eds.): Proc. Seventh Int. Symp. on Modified Cellular and Molecular Controls in Neoplasia. New York, Raven Press, 1976, pp. 279-294.

Phillips, S.M., Stephenson, J.R., Greenberger, J.S., Lane, P.F. and Aaronson, S.A.: Induction of a type C RNA virus by lipopolysaccharide: activity in lipid-A portion upon T and B lymphocytes. J. Immunol. 116: 1123-1128, 1976.

Aaronson, S.A. and Stephenson, J.R.: Intracellular and systemic regulation of biologically distinguishable type C RNA viruses of mouse cells. In Hanna, M.G. and Rapp, F. (Eds.): Contemporary Topics in Immunobiology. New York, Plenum Publishing Corp. In press.

Robbins, K.C., Cabradilla, C.D., Stephenson, J.R. and Aaronson, S.A.: Origin of a B-tropic leukemia virus of the BALB/c mouse strain. Proc. Natl. Acad. Sci. In press.

Peters, R.L., Sass, B., Stephenson, J.R., Al-Ghazzouli, I.K., Hino, S., Donahoe, R.M., Kende, M., Aaronson, S.A. and Kelloff, G.J.: Immunoprevention of X-ray induced leukemias in the C57BL mouse. Proc. Natl. Acad. Sci. In press.

Devare, S.G., Chander, S., Samagh, B.S. and Stephenson, J.R.: Evaluation of radioimmunoprecipitation for detection of bovine leukemia virus infection in domestic cattle. J. Immunol. In press.

Reynolds, F.H., Hanson, C.A., Aaronson, S.A. and Stephenson, J.R.: Type C viral gag gene expression in chicken embryo fibroblasts and avian sarcoma virus transformed mammalian cells. J. Virol. In press.

Tronick, S.R., Golub, M.M., Stephenson, J.R. and Aaronson, S.A.: Distribution and expression in mammals of genes related to an endogenous type C RNA virus of Odocoileus Hemionus. J. Virol. In press.

Stephenson, J.R., Barbacid, M., Tronick, S.R., Hino, S. and Aaronson, S.A.: Proteins of type C RNA tumor viruses. Recent Advances in Cancer Research. In press.

Stephenson, J.R. and Aaronson, S.A.: Differential regulation of endogenous type C viruses of mouse cells. Microbiology. In press.

Stephenson, J.R., Essex, M., Hino, S., Hardy, W.D.,Jr. and Aaronson, S.A.: Feline oncornavirus-associated cell membrane antigen (FOCMA). VII. Distinction between FOCMA and the major virion glycoprotein. Proc. Natl. Acad. Sci. In press.

Essex, M., Cotter, S.M., Sliski, A.H., Hardy, W.D.,Jr., Stephenson. J.R., Aaronson, S.A. and Jarrett, O.: Horizontal transmission of feline leukemia virus under natural conditions in a feline leukemia cluster household. Int. J. Cancer. In press.

Essex, M., Stephenson, J.R., Hardy, W.D.,Jr., Cotter, S.M. and Aaronson, S.A.: Leukemia, lymphoma and fibrosarcoma of cats as models for similar diseases of man. In Hiatt, H.H., Watson, J.D. and Winston, J.A. (Eds.): Origins of Human Cancer. Cold Spring Harbor Laboratories, Cold Spring Harbor,N.Y. In press.

Chander, S., Devare, S.G. and Stephenson, J.R.: Comparison of radioimmuno-assay and Bendixen's hematological evaluation of adult and calf forms of bovine lymphosarcoma. Proc. of Int. Conf. on Bovine Leukemia. In press.

Gardner, M.B., Brown, J.C., Charman, H.P., Stephenson, J.R., Rongey, R.W., Hauser, D.E., Diegmann, F., Howard, E., Dworsky, R., Gilden, R.V. and Huebner, R.J.: FeLV epidemiology in Los Angeles cats: appraisal of detection methods. Int. J. Cancer. In press.

Stephenson, J.R. and Aaronson, S.A.: Endogenous type C viral expression in primates. Nature. In press.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Evolutionary relationships between gag gene-coded proteins of murine and primate endogenous type C RNA viruses. Cell. In press.

Niman, H.L., Stephenson, J.R., Gardner, M.B. and Roy-Burman, P.: Evidence for the possible etiologic involvement of endogenous type C viral gene expression in natural lymphomas of domestic cats. Nature. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04944-02-LRTV

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Determination of Biochemical Abnormalities during Malignant Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. N. M. Papadopoulos, Expert, LRTV, NCI

OTHER: Dr. M. A. Chirigos, Head, Virus and Disease Modification, LRTV, NCI

Dr. J. W. Pearson, Head, Viral Immunotherapy Section, LRTV, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION

Virus and Disease Modification Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
National Cancer Institute, Bethesda, MD 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The determination of the serum proteins of patients with colorectal carcinoma has revealed qualitative and quantitative changes in immunoglobulin levels. New Information was obtained by the demonstration with our electrophoretic fractionation technique of a weak but discrete homogeneous band in the γ-globulin zone in several cases studied. This band has been named minimonoclonal. Our studies of the determination of lactate dehydrogenase-isoenzymes (LD) in normal tissues and serum from abnormal conditions both in humans and in animal models were extended to the determination of LD isoenzyme patterns of the various types of peripheral blood cells. These studies demonstrate that the characteristic pattern of LD-isoenzymes can serve as an indication of the target cell involved in malignancy thus aiding in the diagnosis of the various types of leukemia. The determination of serum lipoprotein patterns was performed in a rare category of type 3 hyperlipoproteinemia (HLP) patients by our unique electrophoretic technique. A finding of major importance was the demonstration for the first time of an extra B-lipoprotein band in the serum of these patients which can be used as a marker to distinguish them from other HLP types by a single electrophoretic test.

Project Description

Objectives:

The adaptation of a sensitive biochemical test system and the development of immunochemical tests for: a) the early detection of biochemical abnormalities before the manifestation of clinical symptoms of disease and, b) the modification of the abnormalities by chemical immunostimulating agents.

The applications of this system of specialized tests in animal models and human studies for gaining information on the humoral mechanism during malignant transformations, on the effects of immunostimulants, and for the search of biochemical markers characteristic of specific abnormalities.

Methods Employed:

A biochemical test system of profile and chemical analysis of the major tissue and cell constituents, proteins, lipoproteins, and isoenzymes, developed by the principal investigator, has been applied for the determination of these constituents in human, animal and tissue culture studies as follows:

1. HUMANS

a) Determination of proteins and immunoglobulins in serum from patients with colorectal carcinoma and control subjects was formed as part of the overall project of "adjuvant therapy in colon carcinoma" in order to gain information on biochemical abnormalities existing before surgery and the changes which may occur after the removal of the tumor and after the administration of immunostimulating agents.

b) Determination of lactate dehydrogenase (LD) isoenzymes in normal tissues and cells to establish a reference library of their characteristic patterns and in serum from patients with defined pathological conditions in order to associate biochemical abnormalities with the sites of their origin.

c) Determination of lipoproteins in serum of a rare category of type 3 hyperlipoproteinemia patients for the study of their electrophoretic patterns.

d) A nipple aspiration technique has been used to obtain breast fluids for biochemical examination. The above mentioned tests have been appropriately modified for the determination of proteins, lipoproteins, and isoenzymes in fluids from control individuals and patients with malignant tumors and benign breast growths.

2. MONKEYS

Determination of serum protein and LD-isoenzyme patterns has been carried out in control monkeys and an experimental group which was subjected to chronic administration of dimethyl hydrazine hydrochloride (DMH) for the induction of colorectal carcinoma. The purpose of this analysis is correlation of biochemical abnormalities detectable by the above tests with histopathologic changes in the tissues of these animals.

3. GUINEA PIGS

A transplantable virus associated mouse leukemia in strain 2 guinea pigs has been utilized as a test model to determine the serum proteins, immunoglobulins and LD-isoenzymes in order to study the biochemical changes occurring during the developmental phase of leukemia and during the remission period after the administration of drugs.

4. MICE

Determination of proteins by electrophoresis and immunoglobulins by immunoelectrophoresis and radial immunodiffusion in the sera of syngeneic and allogeneic mice after; a) Pyran administration, b) MBL-2 inoculation, and, c) MBL-2 inoculation followed by pyran administration.

These experiments are designed to determine the effects of these agents on the hosts and the response of the host as a result of their administration. They form part of a collaborative study of the cellular and humoral mechanisms operating in virus induced cancer.

Progress and Major Findings:

1. HUMAN

a) The determination of proteins in the serum of patients with colorectal carcinoma revealed qualitative and quantitative changes of the immunoglobulins. New information was obtained by the demonstration, with our electrophoretic fractionation technique, of a weak but discrete homogeneous band in the γ-globulin zone, in several of the cases studied. This band is named minimonoclonal.

b) Determination of the component isoenzymes of the enzymes is a sensitive and specific method for the detection of a biochemical abnormality and the site of its origin. Our studies of the determination of lactate dehydrogenase-isoenzymes (LD) in normal tissues and

serum from abnormal conditions both in humans and in animal models were extended to the determination of LD-isoenzyme patterns of the various types of peripheral blood cells. Their distinctive patterns were correlated with serum LD-isoenzyme patterns of patients with lymphatic and granulocytic leukemia. These studies demonstrate that the characteristic pattern of LD-isoenzymes can serve as an indication of the target cell involved in malignancy thus aiding the diagnosis of the various types of leukemia and following its therapeutic course. Part of this work has been published.

c) Determination of serum lipoprotein patterns was performed in a rare category of type 3 hyperlipoproteinemia (HLP) patients by our unique electrophoretic technique. A finding of major importance was the demonstration for the first time of an extra B-lipoprotein band in the serum of these patients which can be used as a marker to distinguish them from the other HLP types by a single electrophoretic test. This work has been accepted for publication.

2. MONKEYS

Biochemical abnormalities were detected in the serum of 4 of the experimental monkeys subjected to the chronic administration of DHM. These studies suggested the performance of a postmortem histopathologic examination of these animals for the search of pathologic lesion in tissues.

3. GUINEA PIGS

Determination of LD-isoenzymes in the serum of guinea pigs after inoculation of the leukemia virus revealed marked changes of the isoenzymes during the acute phase of the disease and a return to normal levels after remission. The fractionation of the serum proteins of the same animals showed a homogeneous band in the γ-globulin zone during remission in the few animals tested.

4. MICE

Determination of globulins, immunoglobulins, and LD-isoenzymes in the serum of mice after the administration of pyran and the inoculation of leukemia virus revealed significant biochemical changes. These preliminary observations suggest further systematic investigations in this test model.

Important Observations from Studies Meriting Special Attention:

1. The demonstration of the presence of minimonoclonal bands in the serum protein patterns of patients with colorectal carcinoma may serve as a biochemical marker; a) for the early detection of the malignancy, and, b) to follow its course after surgery and after the administration of immunoadjuvants.

2. Preliminary findings of biochemical abnormalities in breast fluids determined by our microanalytical test system encourage continued collaboration with a department of surgery engaged in mastectomies.

3. Identification of subjects with type 3 hyperlipoproteinemia by a simple chemical test is of particular importance because they can be successfully treated and they respond to therapy with diet and drugs. Determination of lipoproteins in a family in which the father suffered a heart attack before age 40 revealed that 2 of the 5 children had the extra B band (B-doublet). The serum lipids of these 2 children were within normal levels and their abnormality would have been otherwise undetected. If these observations are confirmed by more studies, the "B-doublet" will serve as a biochemical marker for the early detection of an abnormality, will indicate appropriate treatment, and may aid the prevention of heart disease.

4. It is important to continue the investigation of the mechanism of malignant transformation in the mouse test model because of the extensive experience our section has achieved in this area and the significant biochemical data our specialized test system may contribute.

Publications:

Papadopoulos, N.M.: Clinical applications of lactate dehydrogenase isoenzymes. Ann. of Clin. and Lab. Sci. (in press).

Papadopoulos, N.M., and Herbert, P.N.: The B-Lipoprotein doublet in type 3 hyperlipoproteinemia. Clin. Chem. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04945-02-LRTV

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunologic-Biochemical Responses in Neoplasia Modulated By Adjuvants

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Dr. William A. Stylos, Expert, LRTV, NCI

OTHER: Dr. Michael A. Chirigos, Head, Virus and Disease Modification Section, LRTV, NCI

COOPERATING UNITS (if any)

NONE

LAB/BRANCH Office of Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Virus and Disease Modification Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
National Cancer Institute, Bethesda, MD, 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Purified peritoneal macrophages from allograft-bearing mice were shown by ^{51}Cr release cytotoxicity assays to be specifically cytolytic for the appropriate cells. Maximum reactivity was detected ∿12 days after the S.C. inoculation of allogeneic leukemic cells. I.P. treatment of allograft-bearing mice with pyran acted synergistically to potentiate specific macrophage reactivity. The concentration of different prostaglandins (PGs) was determined in the culture medium of growing BALB/3T3 and virus transformed SV3T3 mouse fibroblasts. The data clearly demonstrates that for cell PGs measured, higher levels were detected in the medium of the SV-40 transformed cells. Of the three series of PGs studied, a 14-fold increase in PGE was detected in the cultures of the SV-40 transformed cells. Supernatants obtained from mouse fibrosarcoma cultures 48 hours after the addition of fresh medium contained a dialyzable material which inhibited the proliferation of syngeneic lymphoma cells in vitro, as measured by ^{3}H-thymidine incorporation. Several lines of evidence indicate that the supernatant inhibitory material is probably PGE. The migration characteristics of the inhibitory substance is the same as authentic PGE as detected by TLC, and indomethacin treatment of fibrosarcoma cultures reduced the amount of supernatant inhibitory substance present.

Project Description

Objectives:

1. By in vitro and in vivo methods, to define which subpopulation of lymphocytes and other cellular components or soluble products are responding to agents known to modulate the immunity of the host.

2. To select agents which act as modulators (at both the cellular and humoral level) of the immune system, and test their efficacy as potentiators in vaccine trials.

3. To elucidate a possible immunologic mode of action for immune modulators by studying the in vivo and in vitro interactions of the cellular components of the immune system with prostaglandins and cyclic nucleotides.

Methods Employed:

A. Standard in vitro immunologic methods will be employed for the detection of the humoral antibody response.

B. Methods for the study of the cellular components of the immune system include:
   1. The use of affinity columns to separate T and B lymphocytes.
   2. Blastogenic experiments using specific T and B cell mitogens in order to determine the relative contribution of the T or B lymphocytes to the overall immune response.
   3. Indirect immunofluorescent studies using specific anti-T or anti-B antiserum to quantitate the relative amounts of T or B lymphocytes participating in the immune response.
   4. ^{51}Cr release for the study of cell-mediated T or B lymphocyte or macrophage-tumor cytotoxicity.
   5. E-rosette assay.
   6. EA-rosette assay.

C. Standard immunochemical methods will be used to isolate and purify tumor-specific soluble membrane antigens.

D. Developmental Methods

In order to study the interaction of the subpopulation of mouse lymphocytes in tumor-bearing mice, the $F(ab)'_2$ portion of polyvalent mouse immunoglobulin and mouse brain cells were used to inject rabbits in order to produce antisera specific for murine T and B lymphocytes. The antibody produced to mouse $F(ab)'_2$ was covalently coupled to cyanogen bromide activated Sephadex G-200. The specificity of the anti-T and anti-B

antisera was determined using appropriately absorbed anti-T and anti-B antiserum and murine T and B lymphocytes separated by an affinity column. Using 3 in vitro assays, i.e., indirect immunofluorescence, ^{51}Cr release, and mitogenic determinants, it was found that 93% of the separated T cells bore T cell antigenic determinants, and 93% of the separated B cells bore surface immunoglobulin receptors.

Tumor-specific surface membrane antigens from L1210 cells have been isolated by 3MKCl extract and by treatment with Triton X-100 detergent. Further isolation and purification will be effected by molecular sieve and ion-exchange columns and by affinity chromatography with cyanogen bromide-activated sepharose columns.

The effect of fibrosarcoma culture supernatant on the proliferation of EL-4 cells was studied by suspending EL-4 cells in varying proportions of medium and supernatant. It was found that the supernatants from the 48 hour culture contain a dialyzable material which inhibits the proliferation of syngeneic lymphoma cells in vitro, as measured by ^{3}H-thymidine incorporation. Preliminary data indicate that the supernatant inhibitory material is a prostaglandin of the E series.

Prostaglandin (PG) levels in cultures of BALB/3T3 and their stable simian virus 40 (SV40) transformed subline were determined by radioimmunoassay with the use of a rabbit antibody directed against a particular PG. Two days after cell inoculation the medium was changed for both cell lines. At 4 days and every 2-day interval up to and including day 16, samples were collected and stored at -20°C. Medium samples were removed from stock solution at each time point and frozen in extraction buffer to determine the PG level of culture medium in the absence of cells.

Major Findings:

The results from in vitro and in vivo studies with the Madison lung carcinoma demonstrate that purified peritoneal macrophages harvested from allograft bearing mice ($CD2F_1$) were shown by ^{51}Cr release cytotoxicity assays to be specifically cytolytic for the appropriate target cells. This cytotoxic release peaked approximately 12 days after subcutaneous inoculation of the allogeneic MBL-2 or L1210 leukemic cells. Intraperitoneal treatment of the allograft-bearing mice with a number of immunopotentiators, notably pyran, acted synergistically to potentiate specific macrophage reactivity.

The concentration of different prostaglandins was determined by radioimmunoassay in both cells and culture medium of growing BALB/3T3 and virus transformed SV3T3 mouse fibroblasts. The majority of the prostaglandins were found in the culture medium rather than in the cells. The data clearly demonstrate that for all prostaglandins measured, higher levels were detected

in the medium of the SV40-transformed cells, as compared with the normal fibroblastic line. Of the three series of PG's studied, a 14-fold increase in PGE was detected in the cultures of the SV40 transformed cell line.

Supernatants obtained from mouse fibrosarcoma cultures 48 hours after the addition of fresh medium contained a dialyzable material which inhibited the proliferation of syngeneic lymphoma cells in vitro , as measured by ^{3}H-thymidine incorporation. Three lines of evidence indicate that the supernatant inhibitory material is probably PGE. First, the supernatant and dialysate of the supernatant contained a substance with the same characteristics as PGE_1 or PGE_2 as detected by thin layer chromatography. Second, PGE_2 treatment of lymphoma cells mimicked the inhibition of proliferation observed with supernatant inhibitory substance. Third, indomethacin (a specific inhibitor of PG synthetase) treatment of fibrosarcoma cultures reduced the amount of supernatant inhibitory substance present.

Preliminary data from short term in vitro studies strongly indicate that T and B lymphocytes from the spleens of tumor-bearing mice undergoing an allogeneic rejection result in dramatic lymphocyte subpopulation changes. These changes were determined by use of specific anti-T and anti-B antisera in an indirect immunofluorescent study and specific T and B cell mitogens to detect blastogenic responses by an increase in the uptake of ^{3}H-thymidine. Five days after the subcutaneous inoculation of 10^{6} MCAs tumor cells there is a slight decrease (not statistically significant) in both T and B cell populations. One week later, there was a slight increase in both T and B lymphocytes from the spleens of the tumor-bearing mice. Twenty-two days after the initial inoculation, there was a statistically significant ($P<0.01$) decrease in both T and B lymphocyte populations. This data indicates that both T and B lymphocytes seem to rise and fall in unison during the allogeneic rejection phenomenon.

Tumor specific soluble membrane antigens from L1210 cells were extracted with either 3MKCl or Triton X-100, and used in conjunction with an immunopotentiator, pyran, in order to augment the specific immune response to L1210. Preliminary in vivo studies indicate that there was a significant rise in the median survival time in the group of mice receiving the Triton X-100 extracted L1210 cells. This data suggests that under optimal conditions it is possible to isolate and purify soluble surface tumor antigens that bear specific tumor antigenic determinants, and further, that it is not necessary to use intact X-irradiated or chemically inactivated whole cells for vaccine studies.

Significance to Biomedical Research and the Program of the Institute:

The use of adjuvants such as BCG and Cornybacterium parvum have been regarded as beneficial immunopotentiators when used in combined modality treatment

of human cancer. Chemicals such as levamisole and pyran, if proven to be effective adjuvants, would be beneficial since they would lack the disadvantages that are inherent in biological agents such as BCG. They can be reproducibly synthesized, quantitated, are devoid of immunogenecity, free of side effects, and can be administered orally. Basic immunologic and chemical studies, both in vivo and in vitro are being conducted with selected active chemical agents in order to attempt to elucidate their mechanisms of action, and to determine their potential use in clinical trials.

Proposed Course:

Preliminary studies from this and other laboratories seem to indicate that certain in vitro assays for the detection of cellular immune responses are exceedingly difficult to standardize and reproduce. Part of this difficulty may be due to the fact that whole tumor cells, and not fragments of tumor cells bearing tumor-specific antigenic determinants, are used in these assays. For this reason, it is proposed to isolate and purify tumor-specific antigens in order to better study the effect of immunopotentiators in modulating the immune system of the host.

Recent experimental data in the field of tumor immunology suggests that as a result of the interaction of certain cellular components of the immune system, soluble mediators are produced that both inhibit and stimulate the immune reaction. One of these substances, prostaglandins, has been strongly incriminated in the initiation of the immune response, and we have found an increased production of prostaglandin E in the supernatant of transformed cells. It is proposed to study what role prostaglandins and certain nucleotides have in the regulation of neoplastic cellular growth control.

Studies will be undertaken to determine which population of lymphocytes undergoes stimulation upon exposure to tumor in vitro. It is becoming more apparent that cell-mediated tumor immunity is due to multiple factor systems including T-lymphocytes, B-lymphocytes, antibody-dependent cell-mediated cytotoxicity, and macrophages. Therefore, any attempt to study the immune reactivity in a tumor-bearing host should include a study of: a) the contribution of each of the cellular and soluble components of the immune system, and, b) the interactions of these components in order to better understand and consequently better control the immuno-reactivity of the tumor-bearing host.

Publications:

Schultz, R.M., Papamatheakis, J.D., Stylos, W.A., and Chirigos, M.A.: Augmentation of specific macrophage-mediated cytotoxicity: correlation with agents which enhance antitumor resistance. Cellular Immunol. 25: 309-316, 1976.

Ritzi, E.M., and Stylos, W.A.: Prostaglandin production in cultures of BALB/3T3 and SV3T3 mouse fibroblasts. J. Natl. Cancer Inst. 56: 529-533, 1976.

Sonis, S.T., Stelos, P., Stylos, W.A., and Wilson, R.E.: Inhibition of lymphoma cell proliferation by supernatant from fibrosarcoma cultures: preliminary evidence that the inhibitory material is prostaglandin E. Prostaglandins. 13: 87-96, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04946-02-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunoprevention of Spontaneously Occurring Tumors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Kende	Expert	LRTV NCI
OTHER:	G. J. Kelloff	Head, Viral Immunology Section	LRTV NCI
	R. J. Huebner	Chief	LRTV NCI

COOPERATING UNITS (if any) B. Sass, Microbiological Associates
I. Al-Ghazzouli, Microbiological Associates
R. Peters, Microbiological Associates
R. Donahoe, Microbiological Associates

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Viral Immunology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) This project has the long range goal of immunoprevention of spontaneous neoplasms in animal model systems that have viral and epidemiological similarities to human neoplasia. To this end, past efforts have included a definition of the endogenous type C viruses and spontaneous tumor expressions in large holding colonies of untreated aging mice and more recently, the project has developed a number of immunoassays to measure the host's natural and induced immune responses to type C viruses and tumor cells, including cellular and humoral cytotoxicity, footpad, lymphocyte transformation, and radioimmunoassays. This project has found that noninfectious viral and virus containing cellular vaccines can be produced which evoke measurable immune responses in the animal and also result in resistance and protection of the animal to challenge with high doses of leukemogenic virus and tumor cells. Studies of the animal's immune response potential versus aging and his endogenous ecotropic and xenotropic type C virus expression (in his tumor cells and his immune system) will provide information to reach the stated goals. The necessary immunoassay and viral assay techniques are now in hand to sort out this complex issue in natural tumor systems and establish the feasibility of these future studies and achievement of the long range goals.

Project Description

Objectives:

(A) Development and utilization of assays for monitoring cell-mediated and humoral immunity in the mouse to type C virus and type C viral antigens and nonviral antigens in cellular vaccines prepared from syngeneic tumor cell lines.

(B) Determination of whether type C virus vaccines or virus containing cellular vaccines can prevent spontaneous, chemically induced, or irradiation induced tumors in mice known to have measurable humoral and cellular immune responsiveness to the viral immunogens.

(C) Determination of the immune competence of laboratory animals with aging and with development of naturally occurring neoplasms and how this relates to endogenous ecotropic and xenotropic virus expression.

Methods Employed and Major Findings:

This effort was established in January of 1976 out of need for an immunology and tissue culture laboratory facility capable of performing humoral and cell-mediated cytotoxicity assays involved in type C virus vaccine immunization studies in the laboratory mouse. Studies this past year have, therefore, involved a comprehensive examination of the structural antigens on the surface of type C virus infected cells and on the type C virus. In these studies cell lines infected with ecotropic (FMR and AKR types) and xenotropic (Class II and Class III) murine leukemia viruses were examined in a complement-dependent ^{51}Cr release cytotoxicity assay with component antisera to the gp70, p12, p15(E) and p30 of Rauscher leukemia virus. Immunoassays with these antisera were also performed on R-MuLV to compare the localization of the proteins on the virus surface with those on the infected cell surface. Gp70 antisera showed predominantly type specificity to R-MuLV, but was shown to cross react with the two classes of xenotropic virus and somewhat less to an AKR type virus from BALB/c mice. P12 antisera showed both type and group reactivity. Cytotoxic activity with p12 antisera was not well correlated with the virus content or the virus producing ability of different R-MuLV shedding mouse cells whereas activity with gp70 antisera was. Viral specific cytotoxic activity was not observed with p30 and p15(E) antisera. Immunoassays of the virion including neutralization with and without complement, virolysis and virus precipitation showed that gp70 and p15(E) were on the viral surface and p12 and p30 were not. The data also established that characteristics of the host cell determine the availability of the nonvirion associated viral antigen to react with antibody on the cell surface.

Having established the cytotoxic activity of monospecific antisera to the type C virus structural proteins, we could then proceed to the humoral cytotoxic immune responsiveness of the mouse to inactivated murine type C

viruses. In these studies inbred mouse strains differing in their endogenous ecotropic and xenotropic type C virus expressions were examined by a complement dependent humoral cytotoxicity assay after immunization with inactivated murine type C viruses. The kinetics of the humoral cytotoxic immune responses was assessed by a ^{51}Cr release assay using target cells of mouse origin highly infected with Rauscher leukemia virus or Gross leukemia virus. All strains of mice responded earlier and more extensively to immunization with inactivated R-MuLV as compared to inactivated G-MuLV. C57BL/6 mice gave higher humoral cytotoxic immune responses than did BALB/c or NIH Swiss mice and were also more reactive in assays measuring resistance to virus challenge and in vitro virus neutralization. The specificity of the humoral cytotoxic immune responses to R-MuLV was evaluated against uninfected or ecotropic or xenotropic murine virus infected target cells or cells of human origin shedding R-MuLV. These studies established that the humoral cytotoxic immune response was specific for type C viruses, and showed cross reactivity among all the known murine type C viruses. A humoral cytotoxic immune response could also be demonstrated in these immunized mice using mouse sera as an endogenous source of complement, suggesting that this immune response may be important in attempts to prevent naturally occurring tumors.

Significance to Biomedical Research and the Program of the Institute:

This project has the long range goal of immunoprevention of spontaneous neoplasms in animal model systems that have viral and epidemiological similarities to human neoplasia. The finding of a low level but ubiquitous incidence of viral antigens in the tumors of low incidence mice coupled with the further findings that these antigens are normally expressed on the cell surface and have specifically been shown to be effective target antigens suggests that all the effector immune mechanisms available to the mouse and the human be evaluated for their responsiveness to these critical viral tumor target antigens. These responses would include antibody mediated cytotoxicity, cell-mediated cytotoxicity, and antibody dependent cell-mediated immunity. This evaluation should occur, at least in part, in experimental protocols where effective immunoprevention has been achieved in vivo.

Proposed Course:

Comprehensive study of the mouse's cell-killing immune responses after immunization with type C virus and type C virus containing immunogen preparations.

Publications:

Peters, R.L., Sass, B., Stephenson, J.R., Al-Ghazzouli, I.K., Hino, S., Donahoe, R.M., Kende, M., Aaronson, S.A. and Kelloff, G.J.: Immunoprevention of X-ray-induced leukemias in the C57BL mouse. Proc. Natl. Acad. Sci. USA, in press.

Kende, M., Sass, B., Donahoe, R.M., Al-Ghazzouli, I.K. and Kelloff, G.J.: Type C virus structural antigens on the surface of the infected cell as determined by a humoral cytotoxicity assay. J. Immunol., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04947-02-LRTV

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

Attempts to Alter and/or Control Tumor Development by Immunological Approaches

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. Klein	Expert	LRTV NCI
OTHER:	J. W. Pearson	Head, Viral Immunotherapy Section	LRTV NCI
	J. Dahlberg	Senior Fellow	LRTV NCI

COOPERATING UNITS (if any)
M. Chirigos, LRTV, NCI
S. Chaparas, DBP, BB
T. Konen, LI, NIAID

LAB/BRANCH Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION Viral Immunotherapy Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Non-specific stumulators (C. parvum and glucan) were employed to enhance the therapeutic response of the L_2C leukemia in strain 2 guinea pigs. Administration of glucan or C. parvum 24 hrs post leukemia inoculation demonstrated an 11 and 9 day increase in median survival time respectively. Prophylactically, only C. parvum was effective in contributing to an increase in survival time. Animals receiving specific active immunotherapy of multiple injections of irradiated autologous L_2C blast cells following chemotherapy were refractory to a viable cell challenge while displaying a corresponding increase in CMI in vitro. Animals treated with chemotherapy alone succumbed to a similar challenge. Treatment of the leukemia following chemotherapy with adoptively transferred syngeneic or allogeneic spleen cells specifically sensitized to L_2C was also successful. Animals receiving syngeneic cells have survived significantly longer than either drug controls or recipients of allogenic cells and are presently still alive. The biological characteristic of a new strain 2 guinea pig stem cell leukemia is described in addition to its growth characteristics and immunogenicity. Employing a spontaneous rat mammary carcinoma, an indirect relationship has been observed between concomitant immunity and metastases.

Project Description

Objectives:

The application of immunotherapy in exploiting antigenic peculiarities of tumor cells is one approach towards eradicating neoplastic diseases via immunological means. This can be accomplished through transfer of immunity with either antibodies or immune lymphocytes or through the stimulation of the immune mechanism in tumor bearing animals employing "immunomodulators." Since the established forms of cancer treatment, namely surgery, chemotherapy and radiotherapy, are generally applicable to the removal of destruction of localized tumor masses and not secondary metastatic outgrowths, collaboration of immunotherapy with these specialties may serve as an adjunct for the elimination of residual tumor cells, thereby improving the overall prognosis.

Methods Employed:

1. Crude membrane extracts prepared from sonicated preps of L_2C and L76 leukemic blast cells with subsequent high speed centrifugation. These extracts are used as solubilized antigen in MIF assays and also for monitoring delayed-type cutaneous hypersensitivity responses in guinea pig tumor systems.

2. Migration inhibition (MIF) and ^{51}Cr release cytotoxicity assays are used to monitor the cell-mediated immune response of pre-sensitized animals prior to or following immunotherapy.

3. A lymphocyte stimulation (LS) assay is used to determine the immunologic competence and memory of lymphocytes upon exposure to various mitogens (i.e., PHA and CON A) and alloantigens. Furthermore, the assay can be used to determine the effect certain types of cells (suppressors) and humoral factors have on blastogenesis or DNA synthesis.

4. Complement-dependent antibody and antibody-dependent cell-cytotoxicity assays are used to investigate and detect the levels of humoral substances in the serum of both tumor bearing and immunized animals.

5. Two additional assays are employed in detecting concentrations of serum antibody at very low levels. One is referred to as a migration inhibition test (MIT) and the other an iodinated binding assay (IBA). Both assays are quite sensitive and can detect nanogram levels of antigen.

6. Nylon wool and rayon wool column fractionation procedures to specifically remove, purify, and isolate T cells from B cells.

7. E and EAC rosetting procedures to characterize and identify B and T cells.

8. Cold phenol extraction of animal tissues to obtain RNA for use in the transfer of biological information to "naive" cells.

Major Findings:

In a recent study, we investigated the possibility of utilizing various immunostimulators in both a prophylactic and therapeutic manner to determine their effect in promoting and augmenting an immune response to an acute lymphoblastic leukemia occurring in strain 2 guinea pigs (i.e., L_2C), keeping in mind potential clinical applicability. Initially, we examined 2 potentiators, Corynebacterium parvum (C. parvum) and glucan, both of which have been reported to produce a generalized increase in immunological reactivity to tumor antigens and both have been used as potent non-specific stimulators of the recticuloendothelial system in animals. Results of these studies indicate that both therapeutic and prophylactic effects were successfully achieved when the potentiators were administered in the presence of the immunogen. Specifically, multiple, bi-weekly, subcutaneous (S.C.) inoculations of x-irradiated L_2C cells mixed with complete Freund's adjuvant and combined with C. parvum induced immunoprophylaxis in the syngeneic host against an L_2C tumor challenge. These results were based upon an increase in survival time and complete protection against tumor growth. A similar approach with glucan, however, was not effective. Therapeutic effect of C. parvum and glucan (administered 1 day after antigen) used alone against the L_2C leukemia resulted in a few long-term survivors. The effects of C. parvum on potentiating the tumor-specific host immune response was also monitored in vitro using a standard macrophage migration inhibition assay and a ^{51}Cr release cytotoxicity assay for measuring both cell and humoral mediated cytotoxicity. Results of in vitro analysis correlated very closely with the immune status of the treated hosts. Those animals which survived a homologous tumor challenge demonstrated both cellular and humoral mediated immunity against the sensitizing immunogen or more specifically, the L_2C tumor-specific transplantation antigens.

Success has also been achieved in the same L_2C system by following chemotherapy with a specific form of active immunotherapy (i.e., adoptive transfer of immunity). Briefly, leukemic strain 2 guinea pigs with white cell counts in the range of 50-80,000/mm^3 were treated with 100 mg cytoxan and 24 hours later received either syngeneic (strain 2) or allogeneic (strain 13 and Hartley) spleen cells from normal and immune animals. Earlier studies had indicated that a dose of 100 mg cytoxan did not completely overwhelm the immune system as revealed by DNCB delayed hypersensitivity responses but was capable of providing a complete remission in leukemic guinea pigs (see report of J. W. Pearson). Results indicated that only those animals receiving immune syngeneic spleen cells specifically sensitized to the L_2C leukemia were protected against tumor growth while animals receiving either normal syngeneic cells or cells from allogeneic sources relapsed at the same time as the drug treated controls (4 week remission).

A semi-in vitro assay (i.e.,WINN assay) was also performed in conjunction with the adoptive transfer experiment. Normal and immune spleen cells from different strains of guinea pigs were incubated with L_2C blast cells

in vitro for 30 minutes at 37°C at a ratio of 200:1 spleen to tumor cells. The mixture of cells was subsequently injected subcutaneously into normal strain 2 animals and tumor growth monitored twice a week. All long-term surviving animals in the above assays were measured periodically for cellular immunity employing a macrophage migration inhibition assay.

WINN assay results revealed that animals receiving immune lymphoid tissue from either a syngeneic or allogeneic source demonstrated a growth inhibitory effect against the tumor with a concomitant increase in survival as compared to animals injected with normal syngeneic or allogeneic cells. More specifically, animals receiving immune syngeneic cells survived the longest, while animals which received Hartley spleen cells were the least responsive to the immune group. Ironically, sera removed from the same immunized donors and measured for immune reactivity to L_2C cells revealed data exactly opposite the cellular responses observed in the WINN assay (i.e., Hartley > strain 13 > strain 2).

Studies have been in progress for the last 6 months in characterizing a new transplantable leukemia which arose in a strain 2 female guinea pig (designated L-76). Employing standard challenge techniques and delayed hypersensitivity reactions, it was determined that this new leukemia shared no cross-reacting tumor-specific transplantation antigens (TSTA) with the established L_2C stem cell leukemia. Cellular immunity to TSTA has up to now been difficult to establish with this new leukemia even following an intradermal injection of irradiated cells in complete Freund's adjuvant. Therefore, the tumor is considered very weakly immunogenic and attempts are currently in progress to immunize and protect guinea pigs against a challenge inoculum.

In contrast to the specific growth pattern of the L_2C leukemia in only strain 2 guinea pigs, the new leukemia has the extended capability of crossing the strain barrier and growing in Hartley and strain 13 animals as well. Additional immunological characterization demonstrated that the L-76 leukemia is also of B-cell origin as is the L_2C leukemia. The L-76 leukemia is transmissible with cellular extracts and produces a widespread disease encompassing the entire hematopoietic system. Morphologic examination of the leukemic guinea pigs showed marked generalized enlargement of the lymph nodes, spleen and Peyer's patches. An incubation period of approximately 12 days is common following a S.C. injection of 10^6 peripheral blood blast cells with very high white blood cell counts (200-300,000/mm^3) observed in the peripheral blood. Further biological characterization of the new leukemia is now in progress including chromosome analysis, pathology and histology of tissue involvement and other immunogenetic markers.

Investigations are currently in progress to study the common antigenicity among viral (e.g., Kirsten sarcoma), chemical (e.g., mammary carcinoma and fibrosarcoma) and spontaneous (L_2C and L-76 leukemias) tumors in guinea pigs. These studies will ultimately attempt to demonstrate the ability of guinea pigs to form antibodies to well-characterized internal and

external (i.e., p30 and gp70) proteins from endogenous and exogenous type C or other reverse transcriptase containing viruses. The 2 assays used for detecting these antibodies include an ^{125}I protein A binding assay which is specific for the Fc portion of an immunoglobulin molecule attached to either cell bound antigens or viral protein and a sensitive radioimmuno-precipitation assay for highly purified type C RNA tumor virus proteins. Sera were developed for each tumor system using multiple injections of x-irradiated cells followed by a live tumor challenge. Animals were bled out, the sera heat-inactivated and stored at -70°C.

We are currently in the process of amplifying the common tumor antigens (TSTA) to the above-mentioned types of tumors in the guinea pig by selective transplantation procedures. Tumors have been selected and tested at various sizes with appropriate immune autologous sera. Those tumors demonstrating the most significant response to their own antisera are subsequently utilized for continuous passage into newborn guinea pigs in hopes of augmenting and perpetuating the antigenic response. Results of in vitro analysis (i.e., ^{125}I binding assay) for both a guinea pig spontaneous mammary carcinoma and fibrosarcoma have indicated that collagenase preparations of tumor cells obtained from 20-25 mm tumors were more responsive to hyperimmune sera than cells obtained from either 5-10, 10-15 or 15-20 mm tumors. This observation was consistent for each subsequent transplant generation. Once antigenic tumors have been obtained via selective transplantation procedures, the tumors and homologous specific antisera will be subjected to assay for cell-surface antigens common to the aforementioned tumors. None of the serums studied have thus far demonstrated complement-dependent cytotoxic antibody. However, non-cytolytic antibodies directed against a number of cell-surface antigens have been detected.

For the past year, this laboratory has been interested in the tumor-host relationship which exists in individuals bearing a progressively growing neoplasm of considerable size. It is a well-established act that tumor bearers can demonstrate a cell-mediated immune response against autologous tumors as evidenced by in vitro lymphocytotoxicity assays. This activity can also be demonstrated in vivo in the form of concomitant immunity in which further grafts of the same tumor are rejected even though the immunizing graft continues to grow. Studies employing a spontaneous mammary carcinoma in the F344 rat has indicated both humoral and cell-mediated concomitant immunity. Host resistance following primary sensitization was expressed most strongly in I.V. and I.P. locations and less strongly in S.C. locations. Tumor cells were seeded in the lungs of normal rats via the tail vein in order to promote artificial metastases to the lung. In order to establish the immune status of tumor-bearing hosts, these animals also received I.V. injections of tumor cells. Results indicated that only tumor bearing animals were resistant to the growth of metastatic nodules in the lung. These results suggest that concomitant immunity plays a significant roll in the control of metastases.

The kinetics of the antibody response in these tumor bearing animals and its relationship to tumor growth is interesting. Increasing amounts of antibody were observed with progressive tumor growth. This antibody response correlated quite well with the degree of concomitant immunity. Animals with larger tumors showed the greatest ability to inhibit the growth of induced lung nodules. An interesting phenomenon associated with this system is the lack of cell-mediated immunity in vitro using spleen cells removed from both tumor bearing and immunized hosts. This inactivity was accredited to the production of suppressor cells. Further studies will examine the ability of sera from tumor bearing animals to convey tumor immunity to normal animals employing adoptive transfer procedures. Peritoneal and alveolar macrophages from tumor-bearers will also be employed in similar protection experiments.

Significance to Biomedical Research and the Program of the Institute:

The current program is directed toward the treatment and control of virus-induced and spontaneous leukemias, carcinomas, and sarcomas in a variety of animal systems. Comparisons made by the use of drugs or surgery alone or in combination with immunostimulators and vaccines may lead to more effective measures.

Proposed Course:

Investigations within the Viral Immunotherapy Section will continue to focus on new conceptual and technical approaches in the study of virus-cell host interactions (Dahlberg), immunological aspects (Klein) and in the prevention and/or control (Pearson) of neoplasia utilizing the guinea pig as a model system.

Biochemical studies will also be undertaken in order to purify and characterize tumor antigens apart from transplantation antigens. We eventually hope to use these purified tumor antigens for two purposes: (1) as a vaccine for various viral, chemical and spontaneous guinea pig tumors in addition to therapeutic application; (2) to demonstrate the existence of common antigens among these tumors through the use of in vivo and in vitro immunological techniques.

In a collaborative effort with other members in our section (Dahlberg and Pearson), immunotherapy studies will continue with the L_2C and L-76 leukemia and other chemically-induced tumors in the strain 2 guinea pig. The use of immune stimulators alone or in combination with syngeneic vaccine preparations will be investigated for their efficacy in treatment.

We have recently initiated studies involving the transfer of tumor-specific immunity with RNA. The RNA will be extracted from immunized allogeneic or xenogeneic sources and transferred in vitro to normal syngeneic tissue. Injections of these immunocompetent spleen cells treated with RNA into tumor

bearing hosts will hopefully prolong survival and perhaps even inhibit tumor growth entirely. We therefore propose to use such an approach as an adjunct to chemotherapy in the treatment of leukemia in guinea pigs. Such treatment could induce or enhance tumor immunity in leukemic animals prior to or during remission. The "immune RNA" has the potential of transferring both cellular and humoral activity and therefore has a distinct advantage over other soluble mediators such as transfer factor. Furthermore, since "immune RNA" can be injected directly or incubated together with autologous lymphocytes, it would obviate the adoptive administration of allogeneic lymphocytes which in turn could result in sensitization to transplantation antigens and subsequent severe graft-versus-host disease.

Publications:

Klein, D.L. and Pearson, J.W.: Modulation of host immune responses against the L_2C guinea pig leukemia. Fed. Proc. (In press.)

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04948-02-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunological Studies of Structural Polypeptides of Type C RNA Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Barbacid	NIH Visiting Fellow, Molecular Biology Section	LRTV	NCI
Other:	S.A. Aaronson	Asst. Chief, Lab. RNA Tumor Viruses	LRTV	NCI
	S. Hino	Expert	LRTV	NCI
	J.M. Krakower	Staff Associate	LRTV	NCI
	K.J. Prozig	Staff Associate	LRTV	NCI
	S.R. Tronick	Microbiologist	LRTV	NCI

COOPERATING UNITS (if any)
R.J. Huebner, LRTV, NCI
K.C. Robbins, Hazleton Lab.
J.S. Greenberger, Harvard Univ.
J.R. Stephenson, LRTV, NCI

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are to 1) purify and immunologically characterize low molecular weight structural polypeptides of mammalian type-C viruses and 2) utilize radioimmunoassays for these polypeptides to search for type-C virus expression in mammalian cells and study the molecular biology of RNA tumor viruses.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To purify and immunologically characterize low molecular weight structural polypeptides of mammalian type-C RNA viruses of different species.

2. To utilize sensitive and specific radioimmunoassays for these viral polypeptides to search for type-C virus expression in cells of different mammalian species including those of primate origin.

3. To study expression of type-C viral antigens in cell lines transformed by sarcoma viruses which are unable to release infectious particles to determine the molecular basis of leukemia-sarcoma virus interactions.

Methods Employed:

Biochemical fractionation procedures including ion exchange chromatography, gel filtration under both denaturing and nondenaturing conditions, and gel electrophoresis. Radioimmunoassays for purified viral structural polypeptides.

Major Findings:

1. The gag gene of mammalian type-C RNA viruses codes for a 65,000-68,000 daltons precursor polypeptide whose complete cleavage leads to the formation of four,p30, p15, p12 and p10, of the five structural proteins of these RNA tumor viruses.

2. The arrangement of the gag gene sequences coding for these four viral structural proteins has been determined as (5')-p15-p12-p30-p10-(3').

3. The major internal antigen p30 as well as two structural proteins, p15 and p10, of all known mammalian type-C RNA viruses were shown to share antigenic determinants. These findings support the concept of a common progenitor in the evolution of type-C RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute:

The applicability of radioimmunologic techniques to the identification and characterization of proteins coded for by the genome of tumor viruses should be of great value in the search for the primary cause of cellular transformation by these viruses.

Proposed Course:

1. Detection and identification of viral proteins in primates tissues from which no infectious viruses have yet been isolated, including human tissues.

2. To study the molecular mechanisms by which these primates exert tight control on infectious virus release but not on the expression of their viral proteins, and correlate them with the incidence of tumors.

Publications:

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Structural polypeptides of mammalian type-C RNA viruses: isolation and immunologic characterization of a low molecular weight polypeptide, p10. J. Biol. Chem. 251: 4859-4866, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: gag gene of mammalian type-C RNA tumor viruses. Nature 262: 554-559, 1976.

Barbacid, M., Stephenson, J.R. and Aaronson, S.A.: Evolutionary relationships between gag gene-coded proteins of murine and primate endogenous type-C RNA viruses. Cell 10: 641-648, 1977.

Krakower, J.M., Barbacid, M. and Aaronson, S.A.: Radioimmunoassay for mammalian type-C viral reverse transcriptase. J. Virol., in press.

Stephenson, J.R., Barbacid, M., Tronick, S.R., Hino, S. and Aaronson, S.A.: Proteins of type-C RNA tumor viruses. In: Recent Advances in Cancer Research, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04950-02-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies on Type-C RNA Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI - S. G. Devare, NIH Postdoctoral Fellow — LRTV NCI

OTHER - J. R. Stephenson, Head, Viral Genetics Section — LRTV NCI
R. K. Reynolds, Microbiologist — LRTV NCI

COOPERATING UNITS (if any)

R. J. Huebner, LRTV	P. Reddy, MA, Beth.	A. Burny, Belgium
P. S. Sarma, LRTV	F. H. Reynolds, FCRC	S. Chander, A.D.R.I., Ottawa
S. A. Aaronson, LRTV	R. V. Gilden, FCRC	M. J. Van Der Maaten, USDA
	S. Oroszlan, FCRC	R. J. Wilsnack, H.R.L., Baltimore

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Viral Genetics Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A highly sensitive and specific radioimmunoassay has been developed for the major structural protein of an oncornavirus etiologically associated with bovine lymphosarcoma. This test can be used to identify cattle which have been exposed to bovine leukemia virus and may thus develop or transmit the disease. Sera from 100% of the cattle with clinically-diagnosed lymphosarcoma exhibited high titered antibodies against the bovine leukemia virus major structural protein. Analysis of randomly obtained sera indicated that infection of this virus is widespread among cattle.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To study the etiologic role of bovine leukemia virus in lymphosarcoma of domestic cattle and to isolate and characterize viral strucutral polypeptides for development of radioimmunologic techniques for detection of viral infection.

Methods Employed:

Biochemical and immunological techniques for isolation and analysis of viral structural polypeptides, radioimmunoassays for detection of viral antigen.

Major Findings:

Development of a radioimmunoassay for detection of bovine leukemia virus infection in domestic cattle: A radioimmunoprecipitation assay for the major 24,000 molecular weight structural proteins of bovine leukemia virus (BLV) has been developed and applied to the detection of BLV infection in domestic cattle. Sera from 100% of adult cattle tested with clinically diagnosed lymphosarcoma exhibited high titered antibody against BLV p24. In contrast, sera from animals with the less common calf form of the disease lacked detectable antibody to BLV p24. The radioimmunoprecipitation assay was shown to have a distinct advantage compared to hematologic criteria, such as specified by the Bendixen's index as well as presently available serologic tests for identification of BLV-infected animals. Analysis of around 6,000 sera from randomly selected cattle from various geographic regions of the United States indicated the presence of antibody against BLV p24 in a high fraction of animals tested. The percentage of animals with detectable antibody to BLV ranged from a low of 2-4% to as high as 42%. These findings indicate that the infection by this virus is much more prevalent among domestic cattle than previously believed.

Significance to Biochemical Research and the Program of the Institute:

A highly sensitive and specific radioimmunoassay has been developed for the major structural protein of an oncornavirus etiologically associated with bovine lymphosarcoma. This test can be used to identify cattle which have been exposed to bovine leukemia virus and may develop or transmit the disease. It is presently not known whether BLV represents potential hazard to humans. However, the interspecies transmission of BLV to sheep through unpasteurized milk has been demonstrated and there has been one report of the development of leukemia in chimpanzees fed milk from lymphosarcoma-positive cows. While the present findings indicate frequent exposure of humans to BLV, sera from 343 individuals in contact with cattle as a result of employment either in slaughterhouses or on a dairy farm, as well as an additional 1,250 human sera obtained from individuals with various forms of cancer all lacked detectable anti-BLV reactivity.

Proposed course:

Studies on the isolation and characterization of viral antigens and development of radioimmunoassays for structural polypeptides of bovine leukemia virus will be continued. These assays will be utilized to study the etiologic role of BLV in spontaneous leukemia of cattle as well as to determine possible hazard to humans.

Publications:

Devare, S. G., Stephenson, J. R., Sarma, P. S., Aaronson, S. A. and Chander, S.: Bovine lymphosarcoma: development of a radioimmunologic technique for detection of the etiologic agent. Science 194: 1428-1430, 1976.

Premkumar-Reddy, E., Devare, S. G., Vasudev, R. and Sarma, P. S.: A simplified radioimmunoassay for viral antigens: use of Staphylococcus aureus as an adsorbent for antigen-antibody complexes. J. Natl. Cancer Inst. (In press, 1977).

Chander, S., Devare, S. G. and Stephenson, J. R.: Comparison of radioimmunoassay and Bendixen's hematological evaluation in adult and calf forms of bovine lymphosarcoma. Proc. of Int. Conf. Bovine Leukemia. (In press, 1976).

Devare, S. G., Chander, S., Samagh, B. S. and Stephenson, J. R.: Evaluation of radioimmunoprecipitation for the detection of bovine leukemia virus infection in domestic cattle. J. Immunol. (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04951-01-LRTV

PERIOD COVERED
July 1, 1976 - September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Role of Endogenous Viruses in Guinea Pig Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	John E. Dahlberg	Sr. Staff Fellow	LRTV NCI
Other:	G. Armstrong	Biologist	LRTV NCI
	J. Pearson	Head, Viral Immunotherapy Section	LRTV NCI
	D. Klein	Expert	LRTV NCI
	B. Barlow	Visiting Fellow	LTVG NCI
	T. Papas	Research Chemist	LTVG NCI
	S. Aaronson	Head, Molecular Biology Section	LRTV NCI
	S. Tronick	Microbiologist	LRTV NCI

COOPERATING UNITS (if any)
K. Perk, Hebrew University, Rehovot, Israel
J. N. Varakis, University of South Alabama School of Medicine
G. M. ZuRhein, University of Wisconsin School of Medicine

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Viral Immunotherapy Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The endogenous viruses of the guinea pig include the B-type GPV, the intracisternal A particle found predominantly in two transplantable leukemias, and the guinea pig herpes virus. We are currently characterizing the structure of GPV, purifying large amounts of virus, and developing probes for the study of levels of GPV RNA and protein synthesis in normal and malignant guinea pig cells. In attempting to immortalize the tumor cells of transplantable guinea pig leukemias, we could show that both herpes saimiri virus and Epstein-Barr Virus (EBV) were infectious for both normal guinea pig lymphocytes and leukemic tumor cells. We also were able to observe and partially characterize two new oncornaviruses by electron microscopy.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04951-01-LRTV

Project Description

Objectives:

(A) To extend our earlier findings that the endogenous virus of the guinea pig is morphologically, biochemically, and immunologically distinct from type C oncornaviruses while it shares properties common to other non-type C viruses such as MMTV and MPMV. We are attempting to develop radioimmune assays and to prepare cDNA probes in order to measure levels of virus protein and nucleic acid synthesis in normal and malignant guinea pig cells, and to search for relatedness between GPV and other non-type C viruses.

(B) To develop a major emphasis on exploring the synergistic potential of non-tumor viruses in conjunction with either non-carcinogenic or carcinogenic drugs, or other viruses, in rapidly transforming guinea pig cells in both *in vitro* and *in vivo* milieus. Primate and rodent herpes viruses are currently being carefully studied as one element vital to this approach.

(C) To continue exploring ultrastructural aspects of virus-cell interactions. These include a continuing interest in virus-like particles which bud from the endoplasmic reticulum, as well as a search among a variety of spontaneous and induced tumors, particularly those of the guinea pig, for signs of latent, chronic, or overt virus infection.

Methods Employed:

Light and electron microscopy, cell culture, virus growth and purification, enzymology, column chromatography, electrophoresis, radioimmune assays, fluorescent antibody, and other preparative analytical procedures.

Major Findings:

(A) (In collaboration with B. Barlow, S. Aaronson, S. Tronick, K. Perk.) During the previous year, a cloned guinea pig cell line was isolated which produced substantial quantities of GPV without any requirement for induction with halogenated pyrimidines. When these cloned cells were exposed to graded concentrations of either dexamethasone or halogenated pyrimidines, no significant increase in virus production, compared to the controls, was observed. This indicated that, in contrast to MMTV production in mouse cells, the glucocorticoid hormones do not affect GPV production. Furthermore, it appears that the level of virus production is at essential maximal levels. This was verified by isolated 54 subclones of this cell line following three cycles of extensive UV irradiation of monolayers of the virus producing clone (designated 118/11). Of the 46 subclones tested for reverse transcription activity, only 4 had enzyme levels higher than the parent cell line. This might well be due to an increase in gene dosage, rather than an alteration of the control mechanisms affecting virus production.

Since virus production at reasonable levels has been possible, an analysis of GPV structural proteins has continued. A summary of the known properties of these proteins is presented in Table 1.

Table 1

Guinea Pig Virus Proteins

Molecular Weight	Remarks	Precipitated by rabbit anti-GPV serum
70000	Major glycoprotein, readily stripped from virion	+
50000	Glycoprotein derived from 70000 dalton glycoprotein by unknown mechanism	+
35000	Phosphoprotein	+
33000-35000	Minor glycoprotein	-
28000	Phosphoprotein	+
23000	Major internal protein	+
18000	Minor protein	-
12000	Phosphoprotein	-

When GPV is rapidly purified by a pelleting through a glycerol cushion, it contains 10-20 times the amount of glycoprotein compared to pelleting and banding overnight in a sucrose gradient. Additionally, if virus is stored at room temperature, or undergoes prolonged centrifugation in sucrose gradients, a variable amount of the 70000 dalton glycoprotein is altered to a form with an apparent molecular weight of 56000 in SDS-acrylamide gels. Whether this represents proteolytic cleavage or loss of sugar moieties is not clear at present. The rapid loss of the major GPV glycoprotein when stored is a reflection of the remarkable overall fragility of GPV virions. When GPV is grown in the presence of radioisotopes and harvested at 4-6 hour intervals, it bands in a sucrose gradient at a density of 1.19 g/cc, identical to the density of MMTV. When frozen and thawed, however, up to half the radioactivity remains at the top of the gradient, and when the virus is incubated for increasing periods of time at 37°C in growth medium, the virus density shifts to 1.17 with increasing levels of degraded material at the top of the gradient. Parallel experiments showed that MMTV underwent no such loss of density or structural components under such conditions.

More recently, we observed that GPV, when grown in the presence of radioactive phosphorus, contains three phosphoproteins. The 12000 dalton protein is probably comparable to the phosphorylated p12 of type C viruses, but the

phosphorylated 35000 and 28000 dalton proteins appear to be unique to GPV. Both these proteins are resistant to ribonuclease digestion, and we are currently studying phosphokinase activity in the virion and will be attempting to explore how these unusual proteins might play a role in virus replication and assembly.

(B) (With G. Armstrong, J. Pearson.) We have recently begun to place a major emphasis on the use of herpes viruses in our analysis of neoplasia in the guinea pig. We are interested in what role, if any, the guinea pig herpes virus (GPHV) might play in naturally occurring neoplasia, and we are also curious about whether suitable manipulation of GPHV in vitro might allow us to transform suitable target cells. Since so little is known about GPHV, our initial studies have been directed to a study of the host range of the virus, development of high titered stocks and a plaque assay, and establishment of suitable serological assays for the virus.

At the same time, in an attempt to determine why the blast cells of two transplantable guinea pig leukemias are impossible to grow in culture, we infected both normal lymphocytes and leukemic lymphoblasts with the primate herpes viruses, herpes saimari, and the transforming varient of the Epstein-Barr virus, B-95-8. Somewhat to our surprise, both HVS and B-95-8 have proven to be infectious for guinea pig lymphocytes, and HVS also is capable of establishing an infection of limited duration (up to 44 days) in epithelial and fibroblastic cells grown as monolayers. HVS infection in lymphocytes also appears to be of limited duration, with antigen expression disappearing between 21 and 28 days post infection, accompanied by an inability to rescue HVS from these cells by co-cultivation on Vero cells or owl monkey kidney cells.

EBV infection of guinea pig cells has been limited to lymphocytes and lymphoblasts, but appears to persist longer, in that antigen is present (at the time of writing) over one month after the initial infection. Uninfected control cells are viable for only a few days, while infected cells remain viable for as long as antigen can be detected.

At this point, there is no evidence that either EBV or HVS is capable of transforming or immortalizing guinea pig cells, although considerable effort will continue to be devoted to this effort.

We have undertaken a series of experiments to determine if inoculation of guinea pigs with either herpes virus or herpes virus infected cells will lead to either an established virus infection or to disease. Although these studies are just underway, it appears that sera from animals inoculated with B-95-8 in utero, and at weekly intervals after birth, contain antibodies to B-95-8. These animals will continue to be monitored for antigen and antibody levels to determine if virus is being produced, or if passive immunization is occurring. Even if only the latter is occurring, the finding is of considerable interest because to date the only sera available for analyzing EBV infections are from human and primate sources.

(C) During the past year we continued to characterize, primarily at the ultrastructural level, a variety of normal and malignant tissues. Our primary aim was to determine whether virus was present in these various samples. In collaboration with J. Pearson and D. Klein, several spontaneously occurring guinea pig tumors were surveyed in the past year. A spontaneous leukemia, designated L-76-1, was shown to contain intracisternal A particles identical to those contained in the widely studied L_2C leukemia (isolated in 1953). Solid tumors, on the other hand, including two mammary adenocarcinomas and a highly malignant sarcoma, were free of detectable virus. When these tumors are grown in culture, they remain free of virus, as measured by RDDP levels, unless treated with halogenated pyrimidines, whereupon they release GPV, as does any other normal or malignant guinea pig cell.

In a collaboration with T. Papas, we were able to show biochemically by the presence of reverse transcriptase and ultrastructurally by the presence of virus-like particles that pike lymphomas contained type C virus. This is an interesting tumor system meriting further study, because the tumors regress during the warm weather and reappear in winter. The properties of the reverse transcription are consistent with the virus playing some role in the etiology of the lymphoma, because the enzyme is most active at $50^{o}C$.

In a collaborative effort with J.N. Varakis and G.M. ZuRhein, we have demonstrated that induction of tumors of neurological origin by inoculation of hamsters with the human papovavirus JC also induces a virus which shares many morphological traits with MMV and GPV. That is, intracytoplasmic A particles appear in the cytoplasm of these tumor cells, and virions band from the cell surface with preformed nucleoids. A feature apparently unique to these hamster virus-like particles is the propensity of intracytoplasmic A particles to band into mitochondria rather than from the plasma membrane. Budding from the plasma membrane is seen only rarely, while virions penetrating mitochondria are very common. At this point, observations are limited to the ultrastructural level, although we are greatly interested in attempting to isolate this unique hamster virus.

Significance to Biomedical Research and the Program of the Institute:

(A) Despite considerable effort by this and other laboratories, type C virions have not been demonstrated in the guinea pig. The only endogenous guinea pig oncornavirus which has been identified is similar to MMTV and other non-type C viruses, and may become a very useful tool to use to look for evidence of viral involvement in guinea pig neoplasia. In characterizing the proteins of GPV, it has become evident that the virus is unusually fragile, with loss of glycoprotein occurring readily with minor perturbations of environment. This fragility is also evident in a density shift and considerable degradation occurring when virus is incubated under relatively mild conditions. A further characteristic which is apparently unique to GPV is the presence of three phosphoproteins, two of which are quite large.

An understanding of the structure of this virus may be a prerequisite to an analysis of the role GPV plays in guinea pig neoplasia, and should ultimately permit us to determine how GPV is related to other oncornaviruses, including viruses which might occur in other South American animals which have not yet been discovered.

(B) Our findings that both HVS and EBV can infect guinea pig cells is of enormous potential value. Although we have not yet been able to determine if guinea pig cells can be transformed by these primate herpesviruses, our evidence is convincing that infection and relatively long-term persistence of the viral genomes occurs. In any case, the ability to infect guinea pig cells with the agent considered, at present, to be the most likely candidate for being a human tumor virus (EBV), and with an agent known to cause tumors in non-human primates (HVS) is an observation of great practical significance. The use of inexpensive inbred rodents to study these viruses permits many experiments to be done which otherwise could either not be done at all, or would be prohibitively expensive. Inbred primates are simply not available and in most cases, the viral background in primates is very high, thus complicating interpretation of results. Inbred guinea pigs will be useful to help sort out the role that the genetic background might play in the etiology of herpes caused disease.

(C) The electron microscope continues to be an invaluable tool in the area of tumor virology. It was only by electron microscopy that the hamster virus present in the JC-induced neurological tumors could be observed. We will continue to be interested in questions of oncornavirus classification, and electron microscopy will remain one of several critical tools in the analysis and classification of newly isolated viruses.

Proposed Course:

(A) A major emphasis will continue to be placed on the growth and purification of high quality GPV. In the next year it is planned that at least one highly sensitive radioimmunoassay will become operational, thus permitting an analysis of GPV protein synthesis in a variety of cells and normal or malignant tissues. Furthermore, it should prove possible to prepare cDNA probes to permit an exploration of GPV RNA and DNA levels in similar cells. At the same time, when these assays are suitably characterized, we will explore the possible relationships of GPV RNA or proteins to other known oncornaviruses, and will initiate a search for related oncornaviruses in wild South American rodents in order to study the evolutionary significance of why GPV and other non-type C viruses appear to be distinct from type C viruses, all of which have, to date, been isolated from Old World species.

(B) We will devote considerable effort to analyzing the nature of EBV and HVS infection of guinea pig cells, and will continue to search for viral transformation, both *in vitro* and *in vivo*. At the same time, additional

effort will be made to work out the fundamental details required in order to work with the guinea pig herpes viruses. All of these herpes viruses will be organized into a program designed to show whether the rate or timing of cellular transformation can be modified by non-oncogenic viruses in combination with each other or with various carcinogenic and non-carcinogenic drugs. At the same time, while guinea pig cells are being insulted by these agents, we will be looking for signs of induction of both type C and type B oncornaviruses.

(C) As guinea pig tumors become available, we will continue to monitor both the original tumor and the cells derived from growth in tissue culture and passage in vivo for evidence of viral involvement.

Publications:

Michalides, R., Schlom, J., Pearson, J., Perk, K. and Dahlberg, J.: Characterization of the oncornavirus particles in the plasma of guinea pigs with the L_2C leukemia. J. Virol. 18: 1120-1130, 1976.

Papas, T., Dahlberg, J. and Sonstegard, R.: Presence of C-type particles in pike lymphoma. Nature 261: 506-509, 1976.

Perk, K. and Dahlberg, J.: Comparative structure analysis of the virus-like particles associated with guinea pig leukemia and the endogenous guinea pig oncornavirus. VI European Congress on Electron Microscopy, pp. 385-387, 1976.

Dahlberg, J.E., Obijeski, J.O. and Korb, J.: Electron microscopy of the segmented RNA genome of La Crosse virus: Absence of circular molecules. J. Virol., in press.

Dahlberg, J.E., Perk, K. and Michalides, R.: Structural and biochemical analysis of virus-like particles present in guinea pigs with L_2C leukemia. Fed. Proc., in press.

Schlom, J., Michalides, R., Perk, K., Pearson, J. and Dahlberg, J.: Biochemical properties of the B-type retravirus of guinea pigs and an agent in the plasma of guinea pigs with L_2C leukemia. Fed. Proc., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04952-01-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Immunologic Studies of Mammalian Type-C Viral Reverse Transcriptases

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI - J. M. Krakower, Staff Associate	LRTV	NCI
Other - S. A. Aaronson, Asst. Chief, Lab RNA Tumor Viruses	LRTV	NCI
M. Barbacid, NIH Visiting Fellow	LRTV	NCI
S. Hino, Expert	LRTV	NCI
K. J. Porzig, Staff Associate	LRTV	NCI
S. R. Tronick, Microbiologist	LRTV	NCI

COOPERATING UNITS (if any)
R. J. Huebner, Lab of RNA Tumor Viruses
K. C. Robbins, Hazleton Lab

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention,
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mammalian type-C viral reverse transcriptases have been purified by ion exchange and affinity chromatography. Sensitive radioimmunoassays, which can detect reverse transcriptase even in the absence of enzymatic function, have been developed. The viral enzyme has been shown to possess type-specific antigenic determinants, as well as determinants shared by all known mammalian type-C viral reverse transcriptases. Thus, rapid type-specific identification of reverse transcriptases in new viral isolates or in tissues harboring type-C viruses is possible.

Reverse transcriptase competition radioimmunoassays have been used to quantitate the enzyme in type-C virus-infected cells. The synthesis and degradation of type-C viral gene products in infected cells is being investigated. These assays should be useful in studying reverse transcriptase expression in mammalian cells possessing endogenous type-C viruses, and in searching for subviral expression of type-C viral components in normal and tumor-bearing tissues of higher primates.

Project Description

Objectives:

1. To purify reverse transcriptases from mammalian type-C viruses.

2. To develop sensitive, type-specific competition radioimmunoassays for these viral enzymes.

3. To utilize competition radioimmunoassays in studying viral gene expression in type-C virus-infected cells.

4. To analyze viral gene expression and regulation in type-C viral mutants.

5. To search for subviral expression of reverse transcriptase in tissues of higher primates.

Methods Employed:

Purification procedures for viral reverse transcriptases include ion exchange and affinity chromatography. Enzymologic methods for detection of DNA polymerase and RNAase H activity are also employed. Immunologic techniques include direct inhibition of viral reverse transcriptase activity by appropriate antisera, and competition radioimmunoassays. Other procedures include gel filtration chromatography and gel electrophoresis.

Major Findings:

1. A rapid purification procedure for reverse transcriptase which preserves native antigenicity has been developed. The reverse transcriptases of Rauscher-MuLV and woolly monkey virus have been purified. Rauscher-MuLV reverse transcriptase has been shown to be a single polypeptide of 80,000 molecular weight, possessing RNA dependent DNA polymerase and RNAase H activities.

2. Sensitive type-, group- and interspecies-specific radioimmunoassays have been developed for known mammalian type-C viral reverse transcriptases. Using these techniques, enzymes from closely related viruses can be distinguished. Furthermore, reverse transcriptases from all mammalian type-C viruses tested have been shown to share interspecific antigenic determinants.

3. In studying viral gene expression in virus-infected cells, the synthesis of the products of different viral genes has been shown to proceed at different rates. The rate of intracellular degradation of viral proteins has also been determined.

Significance to Biomedical Research and the Program of the Institute:

Studies of reverse transcriptase production in cells with endogenous type-C viruses may help clarify cellular mechanisms involved in the regulation of virus gene expression. The availability of a sensitive competition immunoassay which can detect reverse transcriptase independent of enzymatic function provides an additional technique of use in searching for type-C viral proteins in normal and tumor-bearing tissues of higher primates.

Proposed Course:

1. To expand studies to transformed cell lines expressing viral antigens in the absence of infectious viruses, in order to determine the mechanisms involved in cellular regulation of viral genes and to further elucidate the order with the type-C viral genome.

2. To search for reverse transcriptase expression in normal and tumor-bearing tissues of higher primates.

Publications:

Krakower, J. M., Barbacid, M., and Aaronson. S. A.: Radioimmunoassay for mammalian type-C viral reverse transcriptase. J. Virol., In Press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04953-01-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanisms of Tumor Virus Induced Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	K.J. Porzig	Staff Associate	LRTV	NCI
Other:	S.A. Aaronson	Asst. Chief, Lab of RNA Tumor Viruses	LRTV	NCI
	M. Barbacid	NIH Visiting Fellow	LRTV	NCI
	S. Hino	Expert	LRTV	NCI
	J.M. Krakower	Staff Associate	LRTV	NCI
	S. Tronick	Microbiologist	LRTV	NCI

COOPERATING UNITS (if any)
R.J. Huebner, LRTV, NCI
K.C. Robbins, Hazleton Lab.
J.S. Greenberger, Harvard Univ.

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program, Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Naturally occurring RNA-containing sarcoma viruses, that transform fibroblasts in tissue culture, have been isolated from a number of mammalian species. Investigations are being performed to characterize new sarcoma virus isolates with respect to their biological properties and gene products. The properties of sarcoma virus-induced transformants are being compared with tissue culture lines derived from naturally occurring malignancies of human origin to ascertain whether analogies can be found in the mechanisms of neoplastic change.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To study mechanisms of transformation by RNA tumor viruses and to biologically characterize new transforming virus isolates.

2. To study the dominant or recessive nature of the transformed phenotype in somatic cell hybrids involving normal cells and either virus-induced or naturally occurring neoplastic cells.

Methods Employed:

Standard cell biologic and virologic techniques as well as immunological and biochemical assays for detection of transforming virus expression.

Major Findings:

Several previously uncharacterized naturally occurring transforming viruses have been investigated with respect to their biologic properties in tissue culture. Nonproductively transformed cell lines have been obtained and are being investigated immunologically to determine type-C viral proteins coded by different sarcoma viral genomes.

Enzyme-deficient neoplastic cell lines have been selected for use in somatic cell hybridization studies. Several somatic cell hybrids involving neoplastic and normal human cells have been isolated and their malignancy relative to that of parental lines is being tested in immunologically deficient mice. The results should be useful in determining the dominant or recessive nature of the transformed phenotype of different tumor cell types.

Significance to Biomedical Research and the Program of the Institute:

Investigation of the mechanisms of action of naturally occurring transforming viruses may be very important in developing an understanding of the processes leading to neoplastic alteration of human cells.

Proposed Course:

1. To characterize the interactions of transforming viruses with the host cell and develop assays for detection of viral gene products.

2. To determine the dominant or recessive nature of the transformed phenotype of naturally occurring human tumor cells.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04954-01-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Preparation of Large-Size Complementary-DNA from RNA Tumor Virus RNA

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI - Anthony W. Schrecker, Research Chemist, Molecular Biology Section — LRTV NCI

OTHER - S. A. Aaronson, Asst. Chief, Lab RNA Tumor Viruses — LRTV NCI
S. R. Tronick, Microbiologist — LRTV NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to attempt the synthesis of a full-size DNA copy of simian sarcoma virus RNA in an endogenous reverse transcriptase reaction.

PHS-6040
(Rev. 10-76)

Project Description

Objective:

To prepare a complete copy of simian sarcoma virus RNA by a method developed by Baltimore et al. for Moloney murine sarcoma virus RNA.

Methods Employed:

Incubation of simian sarcoma virus with high concentrations of deoxynucleotide triphosphates, one of them radioactively labeled, in presence of salts, detergent and the other usual ingredients of a reverse transcriptase reaction; purification of the labeled DNA formed by SDS-Pronase treatment, phenol extraction, and gel chromatography; sizing of the DNA on alkaline sucrose gradients.

Major Findings:

A small percentage of the DNA formed (approximately 1%) was of the 14S size (approximately 1.1 X 10^6 daltons); this represents about one-half the size of a complete DNA copy of the viral RNA. By contrast, Baltimore et al. obtained a small portion of full-size DNA from Moloney sarcoma virus.

Significance to Biomedical Research and the Program of the Institute:

In the search for viral information in human malignancy, the presence of simian sarcoma virus information in some cases of human leukemia has been postulated. One of the main techniques employed in this search is that of molecular hybridization of the DNA formed in reverse transcriptase reactions to viral RNA. To interpret the results of such hybridizations more accurately, it is desirable to have available a large-size DNA copy.

Proposed Course:

Attempts will be made to prepare a full-size DNA transcript of the viral RNA.

Publications:

Robert-Guroff, M., Schrecker, A. W., Brinkman, B. J. and Gallo, R. C.: DNA polymerase γ of human lymphoblasts. Biochemistry (In press, 1977).

Henderson, G. B., Schrecker, A. W., Smith, C., Gordon, M., Zevely, E. M., Vitols, K. S. and Huennekens, F. M.: Transport of methotrexate and other folate compounds: components, mechanism and regulation by cyclic nucleotides. Adv. Enzyme Regul. (In press, 1977).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04955-01-LRTV

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunological Approaches to the Study of RNA Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI -	S. Hino	Expert	LRTV	NCI
OTHER -	S. A. Aaronson	Asst. Chief, Lab RNA Tumor Viruses	LRTV	NCI
	M. Barbacid	NIH Visiting Fellow, Molecular Biology Section	LRTV	NCI
	J. M. Krakower	Staff Associate	LRTV	NCI
	K. J. Porzig	Staff Associate	LRTV	NCI
	S. R. Tronick	Microbiologist	LRTV	NCI

COOPERATING UNITS (if any)

R. J. Huebner, Lab of RNA Tumor Viruses, NCI
R. E. Wilsnack, Huntingdon Lab
J. R. Stephenson, Head, Viral Genetics Section, NCI
G. J. Kelloff, Lab. of RNA Tumor Viruses, NCI

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of RNA Tumor Viruses

SECTION
Molecular Biology Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goals of this project are to 1) purify the glycoproteins of mammalian type-C viruses, 2) characterize the properties of these proteins biochemically and by radioimmunologic techniques, 3) investigate the expression of endogenous viral gene products in mammalian cells and 4) elucidate cellular mechanisms governing endogenous virus release.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To purify and characterize structural proteins of RNA tumor viruses.

2. To continue development of sensitive and specific immunoassays for newly isolated viral proteins.

3. To analyze the regulation of viral gene expression in host cells or animals.

4. To survey host response to endogenous and exogenous RNA tumor viruses.

Methods Employed:

Standard biochemical methods for protein isolation, such as ion-exchange column chromatography, gel filtration and gel electrophoresis in the presence of sodium dodecyl sulfate; highly sensitive and specific radioimmunoassays using ^{125}I-labelled purified proteins and appropriate antibodies.

Major Findings:

The major envelope glycoproteins (gp70s) of several mammalian type-C and type-D viruses were isolated by means of ion exchange column chromatography from sucrose-banded virion preparations, and radioimmunoassays for these gp70s were developed. Each gp70 was shown to possess a spectrum of antigenic determinants. Type-specific determinants, unique to each gp70 as well as very broadly cross-reactive determinants among many type-C viruses were demonstrable. In typing assays, closely related type-C viruses could be distinguished from each other. By use of an interspecies immunoassay, the first evidence of relatedness of a type-C virus endogenous to primates and a type-D virus (Mason Pfizer monkey virus) was obtained. Thus, these latter findings indicate an evolutionary relationship between type-C and type-D viruses.

Unique antigenic determinants of the gp70s of each class of mouse endogenous type-C virus were found and used to determine which viral genome was expressed in normal mouse tissues and sera. Virus-negative tissues were found to contain gp70 of xenotropic (Class III) endogenous mouse type-C virus. Previous studies have demonstrated gag gene products of this virus also expressed by virus-negative mouse cells. That two genes (gag and env) of the type-C viral genome are coordinately expressed by such cells suggests that cellular restriction to release of this virus must be at a relatively late step.

Antibody response in mice immunized with mouse type-C virus was shown to be predominantly directed against gp70 rather than p30 (major internal protein), whereas immunization of animals with heterologous type-C viruses usually resulted in the reverse. By inducing immunity to the viral structural proteins in mice by either active or passive immunization, the incidence of

radiation-induced leukemia in C57Bl mice was shown to be decreased significantly. In cat lymphosarcoma induced by feline leukemia virus, FOCMA antigen, a target of immunosurveillance, was proved to be unrelated to gp70 or p30. Thus, FOCMA most probably does not represent a structural protein of FeLV.

Significance to Biomedical Research and the Program of the Institute:

Highly sensitive immunological assays with well-controlled specificity are very useful in detecting viral or subviral expression in normal and tumor tissues, in determining their quality and quantity of a given antigen, in classifying a newly isolated virus including putative human viruses, and in surveying the immunologic response to type-C viruses in host animals. In the past year, the sensitivity of these assays has been improved nearly 10-fold and the spectrum of antigens detectable by immunoassays has been widened significantly. These approaches should provide information which may lead to the control of type-C viral tumor induction and/or growth of tumors caused by these viruses.

Proposed Course:

1. Detection and identification of viral proteins in primate tissues from which no infectious viruses have yet been isolated, including human tissues.

2. To study the molecular mechanisms by which these primates exert tight control on infectious virus release but not on the expression of their viral proteins, and correlate them with the incidence of tumors.

Publications:

Hino, S., Stephenson, J. R. and Aaronson, S. A.: Radioimmunoassays for the 70,000 molecular weight glycoproteins of endogenous mouse type C viruses: viral antigen expression in normal mouse tissues. J. Virol. 18: 933-941, 1976.

Aaronson, S. A., Stephenson, J. R., Tronick, S. R. and Hino, S.: Immunological analysis of structural polypeptides of woolly monkey-gibbon ape type C viruses: woolly monkey sarcoma virus. In Clemmesen, J. and Yohn, D. S. (Eds.): Comparative Leukemia Research, Bibl. Haemat., 43. Basel, Karger, 1976, pp. 102-109.

Stephenson, J. R., Hino, S., Garrett, E. W. and Aaronson, S. A.: Immunologic cross-reactivity of Mason-Pfizer monkey virus with type C RNA viruses endogenous to primates. Nature 261: 609-611, 1976.

Stephenson, J. R., Hino, S., Peters, R. L., Donahoe, R. M., Long, L. K., Aaronson, S. A. and Kelloff, G. J.: Natural immunity in mice to structural polypeptides of endogenous type C RNA viruses. J. Virol. 19: 890-898, 1976.

Aaronson, S. A., Stephenson, J. R., Hino, S. and Cabradilla, C.: Endogenous type C RNA viruses of mouse cells: a model for the study of gene regulation in eukaryotes. In Criss, W. E., Ono, T. and Sabine, J. R. (Eds.): Proc. Seventh Int. Symp. on Modified Cellular and Molecular Controls in Neoplasia. New York, Raven Press, 1976, pp. 279-294.

Peters, R. L., Sass, B., Stephenson, J. R., Al-Ghazzouli, I. K., Hino, S., Donahoe, R. M., Kende, M., Aaronson, S. A. and Kelloff, G. J.: Immunoprevention of X-ray induced leukemias in the C57BL mouse. Proc. Natl. Acad. Sci. (In press).

Stephenson, J. R., Barbacid, M., Tronick, S. R., Hino, S. and Aaronson, S. A.: Proteins of type C RNA tumor viruses. Recent Advances in Cancer Research. (In press).

Stephenson, J. R., Essex, M., Hino, S., Hardy, W. D., Jr. and Aaronson, S. A.: Feline oncornavirus-associated cell membrane antigen (FOCMA). VII. Distinction between FOCMA and the major virion glycoprotein. Proc. Natl. Acad. Sci. (In press).

c. LABORATORY OF TUMOR VIRUS GENETICS

July 1, 1976 - September 30, 1977

The major goal of the Laboratory of Tumor Virus Genetics is to develop an approach to the identification of the etiology of neoplasia in both rodents and primates. In order to achieve this goal, appropriate model systems have been developed to identify for the first time a protein responsible for the malignant transformation of a normal cell to a cancer cell and to identify the function of that protein. Several experiments over the past five years have indicated that in a cell transformed by an RNA tumor virus a protein is produced, the continued function of which is absolutely necessary for the maintenance of the transformed state of that cell. The implications of this observation are quite important to designing strategy for detecting etiology, prevention, and possible cure of neoplasia. The first implication is that the continued expression of a viral gene product is necessary for the maintenance of transformation, and the second is that the cancer process itself is potentially a reversible process. Therefore, we have focused our efforts on identifying the protein responsible for this event and the metabolic pathways involved so that in the future we will be able to develop diagnostic tests for such a protein, and define ways to prevent and reverse the effect of such a protein.

The major efforts of this nature that are ongoing in the laboratory are being conducted in the Molecular Virology Section and in the Cellular Transformation Section. In the Molecular Virology Section, the model systems that have been chosen for study involve two types of rapidly transforming RNA tumor viruses isolated from rodents. One type, illustrated by the Kirsten and Harvey strains of murine sarcoma virus are able to transform relatively undifferentiated cells, fibroblasts, in cell culture. The other type, the Friend strain of the spleen focus-forming virus, is able to produce rapid malignancy in hematopoietic cells in vivo but is unable to transform undifferentiated fibroblasts. Therefore these two systems offer an approach to identifying the molecule(s) responsible for the malignant transformation of fibroblasts and other solid tumors, and the identification of the gene product(s) responsible for converting a normal blood cell to a leukemic blood cell.

In the past year, great progress has been made in both of these model systems. Earlier experiments had identified a unique set of rat genetic information responsible for the fibroblast transforming potential of Ki-sv and Ha-sv. In the past year the origin of this rat genetic information has been studied, and for the first time molecule(s) coded for by the rat genetic information, which may be responsible for the

malignant transformation of cells, have been identified. The rat genetic information has been found to be derived from a new type of endogenous type-C virus found ubiquitously in rodent DNA. The elucidation of the origin for this information has established that there are endogenous type-C viruses which are defective for replication as opposed to the classical endogenous type-C viruses that investigators are used to studying. Such defective type-C viruses have been identified in other systems as well, such as mice and rhesus monkeys, and offer a new approach to identifying a human RNA tumor virus. Instead of searching for a replicating type-C virus, which has been the approach taken over the past five to six years, these findings bring forth the concept that human RNA tumor virus may in fact be defective for replication, and therefore channel the human problem into very different approaches for identifying a human virus.

Other studies of the rat oncogenic genetic information and its expression as the cause of various naturally occurring rodent solid tumors have been begun to apply these new virological insights to areas of chemical carcinogenesis. The particular interest in the rat model system for carcinomas and other solid tumors is that several rat solid tumors have been identified which express high levels of the endogenous rat oncogenic genetic information. These cells produce no viral particles but still a viral etiology for these carcinomas seems certain based on a knowledge of the endogenous rat oncogenic information. Since human neoplasia is not apparently associated with a replicating type-C virus, this rat system offers a new and excellent model system for establishing the viral etiology of human cancer. Studies have been undertaken to discern whether this system can be a diagnostic one for the effect of various chemical carcinogens and whether various carcinogens cause the expression of this rat genetic information as their mechanism of action in causing rodent carcinomas. Such a system should allow the distinction between potential vs. actual chemical carcinogens since their effect on oncogenic information can be assessed.

With regard to the coding capacity of the rat genetic information, an in vitro translation system has been developed which has identified for the first time a protein, 21,000 mw, which is coded for by this novel class of rat type-C information. This development opens new areas of investigation in the attempts to identify molecules responsible for the malignant transformation event and find ways to produce antisera against it which could be used both for diagnostic and potentially therapeutic purposes.

In terms of developing ways of identifying the function of viral gene products responsible for transformation, an avian model system has been chosen by the section on Cellular Transformation. The model system is transformation of various cells by the Bryan high titer strain of avian sarcoma virus. The system has the unique advantage that a morphologic marker is present for the type of transformation produced by

this avian sarcoma virus. Vacuoles are produced in these cells transformed by the Bryan virus which closely correlate to the transforming ability of the virus. Since the presence or absence of vacuoles is an easy marker to identify, parameters which effect their presence have been studied. Treatment of cells with deoxyglucose, a compound which inhibits glycoprotein synthesis, suggests that the viral molecule responsible for transformation may be a glycoprotein. If this result remains valid to further testing, study of the membranes of the vacuoles may allow the identification of transforming protein from the avian sarcoma virus as well, and provide useful clues as to where to search for the transforming protein of mammalian sarcoma viruses. Thus by taking a physiological approach in the avian system, and a more chemical approach in the mammalian system, a protein(s) has been identified in the mammalian system which may be responsible for transformation, and a potential locus of its action identified in the avian model system. Thus, the integration of both avian and mammalian model systems has produced useful information in elucidating the viral etiology of neoplasia.

In the area of viral-induced leukemia, as opposed to viral-induced solid tumors, the defective component of the Friend virus has been isolated for the first time. It has been shown to represent a recombinant virus between an ecotropic mouse type-C virus and a xenotropic mouse type-C virus. The finding that the rapidly transforming virus, the Friend spleen focus-forming virus, has acquired endogenous xenotropic viral information reopens the question of the role of endogenous xenotropic viruses in the etiology of leukemia in both rodent and primate species. Since endogenous viruses have been found in Old World primates and in New World species of lower vertebrates, the likelihood that endogenous viral information, which is replication-defective, may be responsible for both solid tumors and leukemias in humans is very high. This does not necessarily imply we have as yet identified such information in human cancer. However, the ubiquitous distribution of defective endogenous type-C information in both rodent and primate species makes the probability exceedingly high that with new approaches such an identification can be made.

In addition two other major areas of investigation are ongoing in the Tumor Virus Genetics Laboratory. The Immunogenetics Section has focused its major effort on understanding the mechanisms of expression of endogenous type-B viral information. The expression of the mouse type-B virus, which is etiologically related to mouse mammary tumors, had been found in 1974 to be stimulated by a glucocorticoid hormone, dexamethasone. In the past year the physiology of the mechanism of action of this glucocorticoid hormone has been elucidated. It has been found that the hormone stimulates the rate of transcription of mammary tumor virus RNA in the absence of new protein or DNA synthesis. This is an important finding for developing new approaches to the use of hormones in treating human cancer and in developing new ways that hormone action can be understood for the eventual application to human cancer. In the future, an

attempt will be made to develop an in vitro assay for the mechanism of action of the hormone, dexamethasone, and to thus identify the cellular components responsible for its action. Identification of the cellular gene products which mediate the hormone action would be an important development in providing new approaches for interrupting the growth stimulating action of hormones in human breast tumors which are responsive to the action of either estrogens or glucocorticoid hormones.

In addition, the Viral Biochemistry Section of the Tumor Virus Genetics Laboratory has been studying the locus which affects the replication of murine type-C viruses, the so-called FV-1 locus. The work in the past year has identified viral protein molecules and the steps in viral replication which are effected by the FV-1 locus. A step in the viral replication cycle which involves either the circularization or integration of viral DNA into host genetic sequences has been identified as the locus of action of the viral target molecules. Future elucidation of the mechanism of action of these molecules could lead to ways of preventing the replication of type-C virus at novel steps in their replication pathway, and thus be useful in the prevention of viral-induced leukemogenesis.

A further approach taken to identify viral molecules involved in transformation has come from the studies on structural viral proteins. It has been established that these are produced from inactive precursor forms by various cellular proteolytic enzymes. One such system studied in great detail in the Laboratory of Tumor Virus Genetics in the past year has been the conversion of the reverse transcriptase of avian myeloblastosis virus from its inactive to active form. The cleavage events have been identified which convert the so-called beta form of the enzyme to the active alpha-beta form and ways of preventing this cleavage are under study. Such molecular studies would be of great use in finding ways to prevent the synthesis of active viral proteins and provide yet another approach to the prevention of viral-induced neoplasia.

In addition to these intramural research activities which span biochemical, cell biological, immunological and genetic techniques, several of the senior investigators within the laboratory spend a substantial amount of their time in support of the Virus Cancer Program. Two of the scientists serve as members of the Coordinating Committee of the Virus Cancer Program, and two others on the Program Resources and Logistics Advisory Group of the Virus Cancer Program. In addition, they and others help provide scientific guidance as project and assistant project officers on various contracts supported by the Virus Cancer Program. In addition, three of the members at the laboratory are on the editorial board of the Journal of Virology, and one is a member of the editorial board of Virology, the two major journals in the field. Thus, the members of the Tumor Virus Genetics Laboratory develop a broad understanding of the cancer problem by close interactions with research that is being conducted outside of the NIH through their editorial board efforts, and through the Virus Cancer Program and its various review groups. Thus, the

members of the Laboratory of Tumor Virus Genetics are in a unique position to attempt to better extramural and intramural research programs by virtue of their combined roles as bench scientists, and their roles in various extramural editorial board committees, and their roles in the Virus Cancer Program.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04835-07-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of the molecular basis of disease by RNA tumor viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. T. Peebles	Sr. Surg.	LTVG NCI
OTHER:	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI
	B. I. Gerwin	Chemist	LTVG NCI

COOPERATING UNITS (if any)

G. Klein, Karolinska Institute, Stockholm, Sweden
J. T. August, Albert Einstein School of Medicine, New York, New York

LAB/BRANCH

Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION

Viral Biochemistry Section

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The molecular basis by which RNA tumor viruses cause disease has been studied and two methods have to date been elucidated: (1) the replicating type-C virus, by being deleted and then recombining with either cell or other viral information, forms a rapidly transforming replication-defective virus. This virus is associated in vivo with sarcomas and leukemias characterized by a short incubation period. The virus is deleted specifically in information for envelope gp70 and core protein polymerase, and sometimes more or less of the gag structural protein: (2) a second method, a model for leukemias of long incubation caused only by nontransforming replicating viruses, is suggested by experiments demonstrating that, if morphologically flat mink cells nonproductively infected by MSV are superinfected by replicating leukemia type viruses, they become transformed concomitant with an increase in sarcoma virus RNA. This would indicate that certain leukemias in animals may occur by increasing, during replication of usually nontransforming leukemia viruses, the levels of RNA from potentially oncogenic but normal cell genes or integrated viral transforming genes.

Project Description

Objectives:

(1) To study cell lines infected with murine sarcoma virus (MSV) in the absence of helper viruses and to characterize the MSV genome in these cells and the MSV genome rescued from these cells.

(2) To search for oncogenic viruses in pediatric tumors.

(3) To develop sensitive biological, immunological, and biochemical assay systems for the detection of possible human tumor viruses.

Methods Employed:

(1) Evidence for sarcoma and leukemia virus expression in cell cultures were measured by known tissue culture assays, biochemical assays, immunological tests, and electron microscopy.

(2) Recently developed mammalian tissue culture detection systems have been utilized in an attempt to find evidence for a viral etiology in pediatric tumors.

(3) Certain tissue culture cell lines are being investigated for their potential use as detection systems for human oncornaviruses using techniques previously employed for investigation of MSV and MuLV.

Major Findings:

(1) Murine sarcoma virus (MSV) defectiveness was studied by examining MSV rescued by RD-114 virus superinfection of both dog and human heterologous host cells nonproductively transformed by the S+L- strain of Moloney MSV (S+L- cells). Biological assays showed that the rescued MSV pseudotype was present in excess over the RD-114 helper virus. The biologically determined virus ratio corresponded to that determined by virus nucleic acid hybridizations. Antisera distinguishing the DNA polymerase of murine type-C virus from that of RD-114 virus demonstrated that the only detectable RNA-dependent DNA polymerase present in the rescued MSV virions was that of RD-114 helper virus. No murine virus polymerase was detectable within the virions.

Previous data have demonstrated no murine-viral polymerase associated with S+L- heterologous human, dog, and mink host cells. The work presented demonstrates that cells producing infectious MSV and helper virions still do not express murine polymerase within released MSV

virions. These findings suggest that heterologous host-cell control mechanisms are not preventing MSV polymerase expression in these cells. Instead, the rescued MSV virions acquire the polymerase protein from the rescuing helper virus. These data provide evidence that the MSV genome is deleted in the information necessary for polymerase, and requires for the production of infectious progeny the functioning of replicating helper virus genes supplying at least viral envelope antigen(s) and virion core protein polymerase.

(2) Using a sarcoma specific probe it was found that, in both mouse and mink cells infected by Moloney sarcoma virus, superinfection of phenotypic revertants of such cells by replicating, but usually nontransforming, mammalian helper viruses leads to increased levels of sarcoma specific RNA in such cells, concomitant induction of foci, and an increase in transformation. This effect can be achieved by either homologous mouse type-C replicating viruses or heterologous viruses capable of replicating in such cells. Whatever the molecular explanation, the results observed in cells exogenously infected with Moloney sarcoma virus and superinfected with the replicating type-C viruses suggests a hypothesis concerning a similar possible phenomenon in cells that might be carrying latent oncogenic information not acquired by exogenous infection. Thus, it is possible that a usually nontransforming type-C virus might act in vivo in a similar manner by leading to an increase in RNA from potentially oncogenic normal cellular or viral genomes during the replication of the nontransforming type-C virus. Such a model would predict that some forms of leukemia or lymphoma caused by type-C viruses (particularly those characterized by a long latent period and involving replicating but nontransforming viruses) might be due to effects on the levels of RNA of cellular genes by the replicating type-C viruses rather than to a direct effect of a transforming gene of the viruses themselves on the growth potential of the cell.

(3) The experiments examining certain pediatric neoplasias in depth for candidate tumor viruses remain in progress. Primary cultures of tumor cells and developed cell lines are being studied.

(4) Certain tissue culture systems are being actively investigated for potential use as detection systems for human oncorna viruses. These experiments remain in progress.

Significance to Biomedical Research and the Program of the Institute:

(1) The exploration of the genetics of mammalian type-C RNA tumor viruses may lead to an understanding of the molecular basis by which these viruses cause disease in host animals. Knowledge of this viral neoplastic process may facilitate the demonstration and eventual understanding of the possible viral-induced cancers of man.

(2) The tumors from pediatric patients are of particular interest for a tumor virologist because of the possibility of vertical transmission of oncogenic viruses. Also clinically, outside of automobile and ingestion accidents, cancer is responsible for most deaths in children in the United States.

(3) Detection and isolation of oncogenic-type viruses from human neoplasia is a necessary first step in demonstrating a viral etiology.

Proposed Course:

(1) We are attempting to further characterize the interactions of the mammalian type-C viruses with their host cells in order to understand the methods by which these viruses disrupt host cell division control mechanisms and induce cancers.

(2) We will continue to attempt to demonstrate a viral etiology in pediatric cancers.

(3) Tissue culture and certain biochemical studies are underway to look for improved methods for possible detection of human oncogenic viruses.

Publications:

Peebles, P. T., Gerwin, B. I. and Scolnick, E. M.: Murine sarcoma virus defectiveness: Serological detection of only helper virus reverse transcriptase in sarcoma virus rescued from nonmurine S+L- cells. Virology 70: 313-323, 1976.

Peebles, P. T., Scolnick, E. M. and Howk, R. S.: Increased sarcoma virus RNA in cells transformed by leukemia viruses: Model for leukemogenesis. Science, 192: 1143-1145, 1976.

Peebles, P. T., Scolnick, E. M. and Howk, R. S.: Detection of sarcoma virus specific RNA transcription in cells transformed by usually non-transforming RNA leukemia type-C viruses: A model for leukemogenesis. Pediatric Research 10: 456, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04846-05-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical analysis of viral infection and its control at the cellular level

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. I. Gerwin	Chemist	LTVG NCI
OTHER:	R. H. Bassin	Head, Viral Biochemistry Section	LTVG NCI
	A. R. Rein	Expert	LTVG NCI
	G. Duran-Troise	Visiting Associate	OD DCCP NCI
	B. Barlow	Visiting Fellow	LTVG NCI

COOPERATING UNITS (if any)
J. Levin, LMG, NICHD

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Viral Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Reverse transcriptase and genomic RNA are not associated catalytically in the core of RNA tumor viruses. Studies of DNA polymerase and virion structural proteins of MuLV nonconditional mutants indicate that reverse transcriptase is not the molecule in a leukemia virus which is inactivated by the Fv-1 restriction system, a naturally occurring cellular system which specifically inhibits the replication of certain murine leukemia viruses. It has been demonstrated that noninfectious MuLV virions lacking reverse transcriptase can abrogate this cellular restriction. Such non-infectious virions should be useful in studying the biochemical mechanism of this abrogation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The goal of this project is an understanding at the molecular level of the life cycle of RNA tumor viruses, i.e., the expression of these viruses in cells infected both nonproductively and productively and cellular control mechanisms applicable to these viruses. The topics of present interest are:

(A) Biochemical characterization of RNA dependent DNA polymerase and virion protein components in infected mammalian cells and noninfectious and/or defective RNA tumor virus particles.

(B) Biochemical studies relevant to mechanisms of cell control of RNA tumor virus replication.

Methods Employed:

(A) Enzymes are purified from disrupted virions and cells using affinity and ion-exchange chromatography and velocity gradients. Enzymes are analyzed physico-chemically by thermolability and/or fingerprint analysis and enzymologically by kinetic constants and/or template preferences. Virion structural proteins are analyzed by SDS gel electrophoresis coupled with immune precipitation.

(B) Various stages in virus replication are studied under conditions where cells do or do not restrict this replication. Studies of early protein synthesis by immune precipitation and gel electrophoresis will be performed.

Major Findings:

(A) (1) Studies of DNA polymerases in RNA tumor virus particles produced by cells treated by actinomycin D have been extended. By comparing the thermolability of DNA polymerase activity in intact control and noninfectious virions it has been shown that the RNA genome does not protect reverse transcriptase in the virion core under conditions where the RNA genome is not degraded. Therefore, the enzyme and its template/primer are not catalytically associated in the virus.

(2) Mutants of MuLV have been found which are noninfectious but can donate to nonrestricted virus sensitivity to a cellular restriction mechanism. It has been shown that these mutants have normal reverse transcriptase function. Other mutants which are similarly noninfectious but cannot donate the restricted phenotype also have normal reverse transcriptase but package large amounts of uncleaved precursor proteins into the virions.

(B) It is clear that noninfectious virions in which reverse transcriptase activity has been destroyed can abrogate cellular restriction mechanisms. Thus these viruses must enter cells and perform some normal viral functions although they cannot give rise to productive infections.

Significance to Biomedical Research and the Program of the Institute:

(A) The relevance of these findings is that a rearrangement of core components must occur for initiation of viral DNA synthesis. Such a rearrangement might provide a control point in the tumor virus life cycle. In addition the mutant studies show that reverse transcriptase is not the molecule which confers sensitivity to the cell restriction mechanism under study.

(B) These studies are relevant for an understanding of cellular control over tumor virus replication. They indicate that noninfectious virions can modify cell restriction mechanisms to enhance the infectivity of other viruses. Such experiments are vital to our understanding of infection by and susceptibility to RNA tumor viruses.

Proposed Course:

(A) The structural protein composition of the mutants will be extensively analyzed. Attempts will be made to correlate certain proteins with demonstrable biological properties of these mutants.

(B) We will attempt to biochemically define early events in RNA tumor virus infection which are restricted by the resistant cell or which can overcome the cellular restriction mechanism.

Publications:

Burstein, S. R., Gerwin, B. I., Taylor, H. and Westley, J.: Formal catalytic mechanism of ascorbate oxidase. Proc. of the First Int. Symposium on Iron and Copper Proteins. Advances in Experimental Biology and Medicine 74: 472-488, 1976.

Rein, A., Kashmiri, S. V. S., Bassin, R. H., Gerwin, B. I. and Duran-Troise, G.: Studies on the mechanism of Fv-1 restriction: Properties of determinants specifying viral tropism. In Animal Virology, D. Baltimore, A. S. Huang and C. F. Fox, (eds.) ICN-UCLA Symposia on Molecular and Cellular Biology IV 269-282, 1976.

Duran-Troise, G., Bassin, R. H., Rein, A. and Gerwin, B. I.: Loss of Fv-1 restriction in BALB/3T3 cells following infection with a single N-tropic murine leukemia virus particle. Cell 10: 479-488, 1977.

Kashmiri, S. V. S., Rein, A., Bassin, R. H., Gerwin, B. I. and Gisselbrecht, S.: Donation of N- or B-tropic phenotype to NB-tropic MuLV during mixed infections. J. Virology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04848-05-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
RNA tumor viruses: Replication, transformation and inhibition in cell cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. H. Bassin	Head, Viral Biochemistry Section	LTVG NCI
OTHER:	B. I. Gerwin	Chemist	LTVG NCI
	A. R. Rein	Expert	LTVG NCI
	G. Duran-Troise	Visiting Associate	OD DCCP NCI
	B. Barlow	Visiting Fellow	LTVG NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Viral Biochemistry Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The major goal of this project is a basic understanding of the biological and biochemical basis of Fv-1 restriction, a naturally occurring cellular system which inhibits the replication of specific N- or B-tropic mouse leukemia viruses. The studies primarily involve a variety of cell culture techniques for quantitation of virus replication and transformation. Current studies emphasize the use of tropic MSV pseudotypes to study Fv-1 restriction independently of MuLV replication.

The Fv-1 related restriction of N-tropic MuLV observed in infectivity studies using Balb/3T3 cells is a result of the requirement for infection with two N-tropic MuLV particles in order for viral replication to take place. The two particles, although identical, have different functions. One particle abrogates Fv-1 restriction in a Balb/3T3 cell rendering it permissive for a period of about 24 hours to productive infection with a second N-tropic MuLV virus.

Quantitative inactivation studies with gamma irradiation and heat indicate that an MuLV particle need not be infectious in order to abrogate Fv-1 restriction.

Project Description

Objectives:

This research project has been largely concerned, during the past year, with a study of the intracellular restriction of N-tropic and B-tropic murine leukemia viruses in mouse cells. This phenomenon, which is known to be controlled by a single cellular gene, Fv-1, is of special interest since an understanding of how restriction operates may be fundamental for studies not only on the mechanisms of cellular control of virus expression but also on the special problems of RNA tumor virus replication and cell transformation.

Methods Employed:

Tissue culture assays including XC plaque assays and focus assays are the main techniques. Development of specialized cell lines which are restricted and permissive for murine viruses. Soft agar focus assays, S+L- plaque assays and immunofluorescence assays for the quantitation of viral infectivity are being used.

Major Findings:

N- or B-tropic pseudotypes of MSV, which are produced following superinfection of mouse S+L- or nonproducer cells with appropriate MuLV's, have been used to study the mechanism of Fv-1^b restriction in Balb/3T3 cells. Results show that infection of a Balb/3T3 cell with a single infectious N-tropic MuLV particle is sufficient to render the cell temporarily permissive to transformation by N-tropic MSV or to productive infection with a second N-tropic MuLV particle. This process has been termed "abrogation of Fv-1 restriction"

Neither B-tropic nor NB-tropic MuLV can abrogate Fv-1 restriction in Balb/3T3 cells. Abrogation of Fv-1 restriction is most effective when the abrogating N-tropic MuLV is added 3 to 24 hours prior to N-tropic MSV, while in the absence of abrogation, N-tropic MSV is completely and irreversibly inactivated by the Fv-1 restriction system within 9 hours after infection.

Our previous data suggested that abrogation of Fv-1 restriction by N-tropic MuLV need not involve normal virus replication. To confirm this idea the kinetics of inactivation of N-tropic MuLV were studied using two procedures, gamma irradiation and heating at 43°C. The ability of N-tropic MuLV to abrogate Fv-1 restriction was found to be much less sensitive to inactivation by gamma irradiation or by heat than was the ability of the same stock of N-tropic MuLV to replicate. Inactivating virus stocks at 43°C for 180 min., for example, results in preparations which are almost fully active in

abrogating activity but possess virtually no infectivity. Thus, abrogation of Fv-1 restriction apparently can take place in the absence of virus replication.

The 2-hit requirement for successful infection of a nonpermissive Balb/3T3 cell is thus a reflection of the requirement for infection with a single non-replicating N-tropic MuLV particle for abrogation of Fv-1 restriction before a second N-tropic MuLV particle is able to replicate with normal efficiency. This 2-hit requirement appears to account completely for Fv-1 restriction of N-tropic MuLV or N-tropic MSV as observed in quantitative XC or MSV focus forming assays in Balb/3T3 cells, although the data must be corrected by an "efficiency factor" when the abrogating virus is not added at the optimal time, i.e., 3 to 6 hours before the second virus.

Previous studies on the nature of the viral determinants which specify N- or B-tropism have also been extended. These studies have shown that MSV can phenotypically acquire tropic determinants from MuLV's during rescue and that mixed infections with N- and B-tropic MuLV's give rise to progeny virus which have phenotypically acquired both sets of determinants and are "dually restricted" for one cycle of infection. Recent studies using NB-tropic MuLV indicate that this virus can also acquire N- or B-tropic determinants from MuLV during mixed infection with the appropriate viruses.

Significance to Biomedical Research and the Program of the Institute:

The Fv-1 restriction system appears to represent a model for intracellular control of RNA tumor virus infection. A detailed study of this system may give us new insights into the control of virus infection in general and RNA tumor virus infection in particular. Once these control mechanisms are fully understood, they could be modified and improved experimentally with possible applications in vivo.

Proposed Course:

Future experiments carried out under this project will be concerned with a biochemical evaluation of Fv-1 restriction and its abrogation by tropic MuLV. Evidence for early synthesis of viral proteins and their relationship to the determinants of viral tropism will be sought. Differences in the pathways leading to viral replication in permissive cells as opposed to abrogation and lack of replication in nonpermissive cells will also be studied. Fv-1 restriction in N type as well as B x N F_1 cells will also be studied by some of the same techniques previously used with Balb/3T3 cells.

Publications:

Rein, A., Kashmiri, S. V. S., Bassin, R. H., Gerwin, B. I. and Duran-Troise, G.: Studies on the mechanism of Fv-1 restriction: Properties of determinants specifying viral tropism. In Animal Virology, D. Baltimore, A. S. Huang and C. F. Fox, (eds.) ICN-UCLA Symposia on Molecular and Cellular Biology IV, 269-282, 1976.

Duran-Troise, G., Bassin, R. H., Rein, A. and Gerwin, B. I.: Loss of Fv-1 restriction in Balb/3T3 cells following infection with a single N-tropic murine leukemia virus particle. Cell 10: 479-488, 1977.

Kashmiri, S. V. S., Rein, A., Bassin, R. H., Gerwin, B. I. and Gisselbrecht, S.: Donation of N- or B-tropic phenotype to NB-tropic MuLV during mixed infections. J. Virology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04858-05-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular studies on the mechanism of transformation of RNA-containing sarcoma viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI
OTHER:	W. P. Parks	Head, Viral Immunogenetics Section	LTVG NCI
	R. Levin	Visiting Fellow	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH: Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION: Molecular Virology Section

INSTITUTE AND LOCATION: Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In vitro translation studies on the RNA prepared from the Harvey strain of murine sarcoma virus have yielded a protein coded for by this defective transforming virus for the first time. The protein has been translated from the sarcoma specific information of the Harvey virus and therefore represents a marker for the first time for potentially transforming information of an RNA tumor virus. This will now enable tumor virologists to purify a protein coded for by a transforming RNA tumor virus which is actually responsible for the change of the normal cell to a malignant cell. The purification and development of assays for such proteins opens new approaches to the identification of the viral cause of neoplasia.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

We have investigated the mechanism of transformation by RNA tumor viruses. The goal is to identify the nucleic acid sequences and protein(s) coded for by RNA tumor viruses responsible for changing a normal cell into a cancer cell. In order to study this phenomenon, we have focused our attention on rapidly transforming, RNA containing sarcoma viruses of mammalian species.

Methods Employed:

(1) Sucrose gradient sedimentation procedures have been used which have allowed the purification of large quantities of the sarcoma virus specific RNA in the absence of contamination with helper leukemia virus RNA.

(2) The preparation of specific cDNA probes from mixtures of sarcoma and helper viruses have allowed us to distinguish in mixtures of viruses the exact biochemical ratios of the helper virus and the sarcoma virus enabling us to purify the sarcoma virus specific RNA and to select for virus mixtures highly enriched for sarcoma virus specific RNA.

(3) The development of a nuclease digested *in vitro* translation system which has an extremely low endogenous background has enabled the sarcoma specific RNA to be translated. This *in vitro* translation system has made possible the identification of the sarcoma virus coded for protein.

(4) Slab gel electrophoresis techniques and autoradiography techniques have been used for the identification of proteins.

(5) The development of various nonproducer cell lines transformed by the replication defective sarcoma viruses in a variety of cell species has greatly facilitated the preparation of the particular viral mixtures.

Major Findings:

(1) The mammalian sarcoma viruses, Kirsten and Harvey strains, have been found to contain a unique set of rat genetic sequences which has become stably associated in covalent linkage with murine leukemia virus information. Abundant evidence has been accumulated that these rat genetic sequences code for the transforming functions of the Kirsten and Harvey sarcoma virus. A procedure was developed over the past year in order to obtain large quantities of Harvey and Kirsten viral specific RNA in the absence of contaminating helper virus RNA. This was necessary in order

to develop in in vitro translation systems which might yield sarcoma virus specific protein(s). The fact that heterodimers do not form between helper virus and sarcoma virus RNA has enabled us to develop specialized sucrose gradient sedimentation procedures to separate the viral RNAs and precluded the necessity of separating the RNAs on acrylamide gels which would have made obtaining the large quantities of RNA difficult that were necessary for performing the experiments.

(2) Utilizing the sarcoma specific RNA we have developed in vitro translation systems utilizing mouse cells which respond to the sarcoma virus RNA. In this in vitro translation system a protein has been synthesized which is faithfully initiated and which is 21,000 molecular weight. A similar protein has been obtained from both Kirsten sarcoma virus and Harvey sarcoma virus. This protein is not coded for by helper virus used to pseudotype sarcoma virus in either case and thus represents a sarcoma virus specific protein obtained for the first time.

(3) In addition to this work on the Kirsten and Harvey sarcoma virus, we have obtained a cDNA probe for the first time specific for the sarcoma virus specific sequences of a primate sarcoma virus, namely the woolly monkey sarcoma virus. With the probe specific for the transforming information of a primate sarcoma virus we are now in a position to search in primate tumors with a different type of cDNA probe than has been previously available in order to search for the presence of sarcoma specific information in primate tumors.

Significance to Biomedical Research and the Program of the Institute:

The ability for the first time to detect a protein potentially responsible for the transformation of cells represents a unique step forward in ultimately identifying the etiology for cancer. All previous assays for proteins coded for by RNA tumor viruses have relied on the detection of structural proteins of the viruses and not proteins actually responsible for either the leukemic genecity or the transforming ability of a given RNA tumor virus. The identification of a marker for a transforming protein from an RNA tumor virus from any species for the first time represents a milestone in the approach to the identification of the etiology of either rodent tumors or human tumors since it will be possible in the future to search for a completely new class of proteins, namely those actually responsible for the malignant transformation of cells.

Proposed Course:

The protein that can be synthesized with radioactivity in vitro will be used as a marker to purify an equivalent protein in cells transformed by

RNA tumor virus. In addition, it will allow the preparation of antisera which can be identified by their ability to immunoprecipitate the radioactive protein. Furthermore the protein can be sequenced from its N-terminal end in an attempt to provide the sequence of the protein in order to synthesize large quantities of proteins to immunize animals with and prepare antisera.

Publications:

Scolnick, E. M., Williams, D., Maryak, J., Vass, W., Goldberg, R. J. and Parks, W. P.: Characterization of the coding capacity of endogenous sarcoma virus specific RNA. J. Virol. 20: 570-582, 1976.

Ruscetti, S., Scolnick, E. M. and Parks, W. P.: Natural immunity in cats to feline leukemia viral antigens. J. Immunol. 117: 2029-2035,1976.

Levin, R., Ruscetti, S. K., Parks, W. P., and Scolnick, E. M.: Expression of feline type-C virus in normal and tumor tissues of the domestic cat. Int. J. Cancer 18: 661-671, 1976.

Young, H. A., Shih, T. Y., Scolnick, E. M. and Parks, W. P.: Steroid induction of mouse mammary tumor virus: Effect upon synthesis and degradation of viral RNA. J. Virol. 21: 139-146, 1977.

Scolnick, E. M. Williams, D. and Parks, W. P.: Purification and characterization of the viral RNA of a sarcoma virus isolated from a woolly monkey. Nature 264: 23-30, 1976.

Troxler, D. H., Parks, W. P., Vass, W. C. and Scolnick, E. M.: Isolation of a fibroblast nonproducer cell line containing the Friend strain of the spleen focus-forming virus. Virology 76: 602-615, 1977.

Shih, T. Y., Young, H. A., Parks, W. P. and Scolnick, E. M.: In Vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin: Elongation of chromatin associated RNA by E. coli RNA polymerase. Biochemistry (in press).

Parks, W. P., and Scolnick, E. M.: In Vitro translation of Harvey Murine Sarcoma viral ribonucleic acid. J. Virol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04899-05-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mechanism of AMV DNA polymerase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. S. Papas	Chemist	LTVG, NCI
OTHER:	M. Chen	Visiting Fellow	LTVG, NCI
	D. J. Marciani	Expert	LTVG, NCI
	J. P. Bader	Head, Cell. Transformation Section	LTVG, NCI
	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG, NCI
	R. Goldberg	Microbiologist	OADVO, NCI

COOPERATING UNITS (if any)
J. G. Chirikjian, Georgetown School of Med., Wash., D.C.
W. J. Martin, Food and Drug Adm.
R. Axel, Columbia U., N.Y.
S. Wilson, LB, NCI
P. Sarin, LTGR, NCI
R. A. Sonstegard, Univ. of Guelph, Canada

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Cellular Transformation Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The mechanism of release of α subunit from dimeric αβ AMV DNA polymerase was elucidated in great detail. Functional differences between the RNase H activity of α and αβ forms were determined by immunological data. Antisera against α and αβ forms inhibit the DNA polymerase activity of both forms but only the RNase H activity of the enzyme form used as immunogen. Antibody specifically inhibitory for αβ RNase H activity can be absorbed by purified α, and antibody specifically inhibitory for α RNase H activity can be absorbed by purified αβ. These data indicate that antibodies against α and αβ forms of AMV reverse transcriptase recognize distinct antigenic determinants present on α and that the α and αβ forms differ in the steric relationship of these determinants to the RNase H active site. Active site directed reagent (pyridoxal 5'-phosphate) revealed that the deoxynucleoside triphosphate binding site is located on the α subunit. The α subunit possesses five reactive amino groups, one of which is essential for catalytic activity; the β subunit has three reactive amino groups which are not involved in the deoxynucleoside binding site.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The scope of this investigation is aimed at contributing to the understanding of the mechanism of viral replication with an emphasis on the eventual understanding of cell transformation by RNA tumor viruses. The study of the subunits and activities of the reverse transcriptase will be used to study viral replication.

Methods Employed:

(A) AMV DNA polymerase was prepared in large quantities by utilizing pyran-sepharose affinity fractionation and elution with specific templates. This process combines affinity fractionation and affinity elution. The resulting protein appears to be homogenous. Such state of purity is prerequisite to most of our studies.

(B) Solid phase treatment of precursors by carboxymethylcellulose-bound trypsin.

(C) Chemical modification of viral RNA by chloroacetaldehyde.

(D) Immune precipitation utilizing ^{14}C alkylated polymerase, and analysis by SDS gel electrophoresis.

(E) Purification of kinases from transformed cells and RNA tumor viruses by ATP-sepharose affinity chromatography.

(F) *In vitro* phosphorylation of viral structural proteins and AMV DNA polymerase.

(G) Molecular weight determination of nucleic acid by electrophoresis in formamide under fully denatured conditions on exponential polyacrylamide gels.

Major Findings:

(A) The reverse transcriptase of avian myeloblastosis virus (AMV) exists as a dimer of two polypeptides or as a single polypeptide, designated $\alpha\beta$ and α, respectively. Both the α and $\alpha\beta$ forms of the enzyme possess DNA polymerase and RNase H activities (EC 3.1.4.34). Antisera raised in rabbits against the α and $\alpha\beta$ forms inhibit the DNA polymerase activity of both forms but only the RNase H activity of the enzyme form used as immunogen. Antibody specifically inhibitory for $\alpha\beta$ RNase H activity can

be absorbed by purified α, and antibody specifically inhibitory for α RNase H activity can be absorbed by purified αβ. These data indicate that antibodies against α and αβ forms recognize distinct antigenic determinants present on α and that the α and αβ forms differ in the steric relationship of these determinants to the RNase H active site. These results are consistent with possible functional differences between the RNase H activity of the α and αβ forms of AMV reverse transcriptase.

(B) Primary structure for $tRNA^{Trp}_{beef}$ and primer $tRNA^{Trp}_{avian}$ show only minor differences in sequences. The heterologous $tRNA^{Trp}_{beef}$ appears to have similar properties in its ability to bind the αβ form of AMV DNA polymerase. A molecular weight of 190,000± 10,000 was obtained for the enzyme-tRNA complex suggesting a 1:1 interaction. In addition, $tRNA^{Trp}_{beef}$ can hybridize to the avian 35S viral RNA as well as act as a primer for its transcription. Unlike the avian primer, $tRNA^{Trp}_{beef}$ can be obtained in substrate amounts for future studies dealing with interactions between the two macromolecules.

(C) Avian myeloblastosis virus (AMV) DNA polymerase is inactivated by preincubation with pyridoxal 5'-phosphate. This inactivation is relatively specific since various pyridoxal 5'-P analogs cause no inactivation. This effect is reversible but can be made irreversible by reduction with sodium borohydride; the reduced pyridoxal-5'-P adduct exhibits a new absorbance maximum at 325 nm and a fluorescence emission at 392 nm when excited at 325 nm. The evidence presented suggests the formation of a Schiff base between pyridoxal-5'-P and a nucleophilic residue of AMV DNA polymerase. The presence of a deoxynucleoside 5'-triphosphate (dTT) protected the enzyme from inactivation. Reduction of the pyridoxal-5'-P enzyme complex in the presence or absence of a deoxynucleoside 5'-triphosphate showed that the α subunit possess five reactive amino groups, one of which is essential for the catalytic activity; the β subunit has three reactive amino groups which are not involved in the deoxynucleoside binding site.

(D) Storage of the dimeric (αβ) form of avian myeloblastosis virus (AMV) DNA polymerase in glycerol resulted in the release of the smaller α subunit, as detected by glycerol gradient sedimentation. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of enzyme stored in glycerol showed the concomitant appearance of several polypeptides and a lowering in the level of both β and α components. This reduction appears to be the result of cleavages introduced by traces of hydrolytic activity present in glycerol samples. An enhancement of α subunit released, as detected by activity profile, was also achieved upon direct but limited exposure of purified avian myeloblastosis virus DNA polymerase to carboxymethyl-cellulose-bound trypsin matrix. Electrophoretic analysis of digested enzyme revealed a progressive fragmentation, with simultaneous increase in the α subunit and decrease in the β subunit.

(E) Northern pike lymphosarcoma DNA polymerase was partially purified from particulate fractions banding at 1.15-1.16 gm/cc^3 from homogenates prepared from frozen necropsies of tumor-bearing pike. The enzyme behaves

as a typical reverse transcriptase in that it prefers ribotemplates to deoxytemplates. The isoelectric point (Ip 5.5) is similar to AMV polymerase. The pike enzyme elutes from phosphocellulose column at 0.22 M salt concentration; the same as native AMV polymerase. The enzyme activity is inhibited by pyran, a specific inhibitor of viral DNA polymerases. The enzyme has ribonuclease H activity which is known to be present in retrovirus DNA polymerases. The most striking difference between the pike lymphoma polymerase with the other viral DNA polymerases tested is the low Tmax of 20°C compared to 30°C for RLV polymerase and 38°C for AMV and RSV. The Tm studies suggest that evolutionary pressures on retraviruses from widely divergent classes of vertebrates (fish, birds, and mammals) have selected Tm profiles lower than the known host DNA polymerases. The correlation of the Tm of the reverse transcriptase to the ambient temperature of the viral host suggests a long association of the virus to the host or possibly that these enzymes are host derived.

Significance to Biomedical Research and the Program of the Institute:

It is clear that the formation, the maintenance, and the expression of pro-virus are the central features of the life cycle of RNA tumor viruses. Many questions related to these aspects are unsettled. Elucidation of the nature of the enzymes and other factors involved in each step of viral replication and of their mechanism of action will be essential to gain an understanding of the process of oncogenesis induced both by exogenous and endogenous viruses. The availability of DNA provirus will contribute to our knowledge of the life cycle of the oncorna viruses and will accelerate restriction mapping and virus genome integration.

Proposed Course:

(A) To elucidate the detailed mechanism and mode of catalysis of AMV DNA polymerase. Elucidate primer nucleotide sequences essential for specific binding to the polymerase using purified tRNA's having sequences similar or different than $tRNA^{Trp}$ primer.

(B) To translate 35S AMV RNA using various *in vitro* translational systems. *In vitro* synthesis of "gag protein" and AMV polymerase.

(C) To identify precursors of AMV DNA polymerase and study the mechanism of cleavage to active enzyme molecules. Isolate and characterize membrane bound host or viral proteases responsible for specific cleavage.

(D) Investigate in detail the mechanism of circularization of 35S RNA in a reconstituted system with heterologous primer $tRNA^{Trp}_{beef}$ and in turn determine its effect in the synthesis of long cDNA transcripts.

(E) To investigate the effect of factors which stimulate polymerase activity on the size of cDNA transcripts. Establish conditions that increase the yield of large cDNA transcripts.

Publications:

Papas, T. S., Marciani, D. J., Samuel, K. and Chirikjian, J. G.: Mechanism of release of active α subunit for dimeric αβ avian myeloblastosis virus DNA polymerase. J. Virology 18: 904-910, 1976.

Papas, T. S., Dahlberg, J. E. and Sonstegard, R. A.: Type C virus in lymphosarcoma in northern pike (Esox lucius). Nature 261: 506-508, 1976.

Papas, T. S., Renzi, G. R. and Martin, J. W.: Immunological distinction between ribonuclease H activity of α and αβ forms of avian myeloblastosis virus (AMV) reverse transcriptase. Virology (in press).

Papas, T. S., Pry, T. W. and Marciani, D. J.: Inactivation of avian myeloblastosis virus DNA polymerase by specific binding of pyridoxal-5'-phosphate to deoxynucleoside triphosphate binding site. J. Biol. Chem. (in press).

Baroudy, B. M., Papas, T. S. and Chirikjian, J. G.: Binding of $tRNA^{Trp}_{beef}$ to AMV DNA polymerase and its function as a heterologous primer. Proc. Natl. Acad. Sci. (USA) (in press).

Papas, T. S., Pry, T. W., Schafer, M. P. and Sonstegard, R. A.: Presence of DNA polymerase in lymphosarcoma in northern pike (Esox lucius). Cancer Research (in press).

Sonstegard, R. A. and Papas, T. S.: Descriptive and comparative studies of type C virus DNA polymerase of fish. Amer. Assn. Cancer Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04928-05-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transcriptional regulation of the expression of RNA-containing tumor virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. P. Parks	Head, Viral Immunogenetics Sec.	LTVG, NCI
OTHER:	R. G. Wolford	Microbiologist	LTVG, NCI
	M. C. Noon	Biologist	LTVG, NCI
	E. Hubbell	Microbiologist	LTVG, NCI
	C. Chen	Biologist	LTVG, NCI

COOPERATING UNITS (if any)

E. M. Scolnick, LTVG, NCI
H. A. Young, LTVG, NCI
W. Heston, LBV, DCB, NCI
T. Y. Shih, Expert, LTVG, NCI
Project No. Z01-CP-04963-02-LTVG
H. Meier, Jackson Laboratories

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION

Viral Immunogenetics Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	2.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies of transcriptional regulation of inherited viral genes provides an approach to learn how cells control expression of various genes involved in cancer. Murine mammary tumor virus expression in both cell culture and in whole animals has been used to study the genetic and non-genetic influences governing transcriptional regulation. Glucocorticoids stimulate the rate of viral RNA synthesis in cell culture. To investigate this process further, attempts have been made to utilize various methods for detecting newly synthesized RNA in cell-free systems. We conclude that further understanding of transcriptional regulation requires a detailed understanding of the primary structure of the viral nucleic acid. The relevance of regulation of mammary tumor virus expression to natural mammary adenocarcinoma in mice was shown by the high correlation between MMTV milk virus titer and the propensity to develop tumors. In multiple second backcross females between high and low incidence mouse strains, simple Mendelian genetic influences could not be demonstrated because of the multifactorial etiology of the mammary tumors.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Overall - an understanding of genetic and nongenetic factors which influence levels of virus expression.

(1) Development and application of quantitative techniques for measurement of *in vitro* viral RNA transcription.

(2) Isolation of closed circular MMTV double-stranded DNA molecules for insertion into pCR-1 for molecular cloning.

(3) Isolation and characterization of naturally occurring cell variants with widely different levels of virus expression. Utilization of virus isolates from related *Mus* species to attempt detection of transforming sequences in mammary tumors.

(4) Conclusive genetic data that mouse mammary tumor virus expression is naturally genetically determined.

(5) Extension of regulatory studies to human mammary tumor cell in cell culture.

Methods Employed:

(1) Quantitative biochemical techniques for measuring viral RNA transcripts from DNA include the *in vivo* (whole cell) assay of Coffin *et al.* Pulse-labeled radioactive (^{3}H-uridine) viral-specific RNA is "fished out" from the total cellular RNA transcript by successive hybridzation to dC-elongated viral cDNA and then to poly I-sephadex. This technique allows measurement of a virus-specific fraction of 0.1 - 1.0% of the newly synthesized RNA, the usual level of viral RNA synthesis in viral-infected cells.

An *in vitro* assay utilizes mercuriated UTP as a precursor to new RNA synthesis. In this assay, the RNA is then recovered from sulfhydral affinity columns and assayed by nucleic acid hybridization using radioactive viral cDNA probes.

(2) Recombinant DNA technologies involve the large scale isolation of form I mouse mammary tumor virus (MMTV) covalently closed circular

double-stranded DNA molecules. These forms exist in the nucleus of acutely infected or chronically MMTV producing cells. The molecule is then cleaved in one place with a restriction enzyme RI, thus creating a linear double-stranded DNA which can then be joined to a plasmid and used to transform an E. coli host, 1776, approved for use in this type of study. This technology requires p4 level containment and utilization of Class III cabinets.

(3) Recombinant inbred lines involved the crossing of two inbred strains of mice to produce hybrid offspring, the F1 generation. From the F1, brother-sister pairs (approximately 20) are selected and matings are performed within each lineage for 20 successive generations. This technology fixes a wide range of parental characteristics and is particularly well-suited to the study of multifactorial systems. A high mammary tumor incidence strain, C3H/HeJax, and a low incidence strain C57L/Jax, were used in collaboration with Dr. Hans Meier to construct 16 different RI lines from this cross.

Major Findings:

(1) The use of an in vivo assay described by Coffin et al.to measure newly synthesized viral RNA revealed that glucocorticoids stimulated the rate of MMTV RNA synthesis by 10 minutes after hormone addition. The rate of MMTV RNA synthesis increased for approximately 60 minutes and reached a rate 10 times greater than that measured in untreated cells. At maximal rates, between 1.0 and 3.0 percent of newly synthesized cellular RNA represented transcription from the MMTV DNA sequences. There was no detectable effect on type-C virus RNA synthesis in these experiments indicating that there is a specific nucleic acid sequence recognition signal involved in hormone stimulation. The rapidity, magnitude and high degree of specificity of MMTV stimulation by glucocorticoids indicates a primary effect on transcription. This is the first demonstration of mechanism of glucocorticoid action and exemplifies the use of a virus to study a very common process in mammalian cell regulation.

(2) Previous attempts in our laboratory to reduce the whole cell to smaller transcriptional units have significantly altered complex intracellular regulatory processes. Thus, the reported biologic faithfullness of the mercuriated UTP assay offered considerable promise to extend our understanding of the mechanism of glucocorticoid stimulation of MMTV RNA synthesis. Studies revealed however that the assay primarily measures pre-existing RNA levels and not newly synthesized RNA templated from DNA. These results suggest strongly that the relatively low levels of RNA transcription noted in viral systems (0.1 - 1%) are not amenable to molecular analysis with current technologies in either whole cell or chromatin systems.

(3) As an alternative approach to fractionating whole cells to prepare transcriptional systems, we are isolating MMTV DNA and pursuing a fundamentally different approach. This approach is predicated on a detailed understanding of the structure of the MMTV nucleic acid. MMTV should contain not only ribosome binding sites, promotion and termination signals, but also RNA binding sites and perhaps a site that will bind the glucocorticoid-receptor complex. So far the preparation of form I MMTV DNA indicates either chronically or acutely infected cells can be used as a source of DNA for molecular cloning and amplification in E. coli.

(4) Mammary tumor cell lines from spontaneous tumors occurring in RIII mice have been prepared. These cells are epithelioid in appearance, and surprisingly express only low levels of MMTV specific RNA and polypeptides. Using cDNA from a related retrovirus from Mus cervicolor, expression of a novel class of viral-related sequences was detected. These virus related sequences are not incorporated into virus particles and their function is unknown. They are being investigated as putative epithelial transforming sequences analogous to fibroblast 'sarc' sequences.

(5) A high correlation between mammary tumor virus expression and mammary tumor incidence was shown in various backcrosses between GR, a high mammary tumor incidence strain and C57Bl/He, a low incidence strain. The segregation of tumors strongly suggested in most backcrosses that the genetically transmitted MMTV provirus was responsible for tumorigenesis since either parent effectively transmitted the genotype responsible for the mammary tumor phenotype. The segregation pattern resembles a single Mendalian dominant gene but in second backcross segregants the influence of multiple genes became obvious. Thus, the factors governing mammary tumorigenesis are highly complex but are highly correlated with factors which regulate the expression of mammary tumor virus expression.

The genetic complexity is so great that even RI lines have failed to provide adequate segregation of the major genetic influences.

(6) Several human cell lines from malignant human mammary adenocarcinoma have been established.

Significance to Biomedical Research and the Program of the Institute:

The regulation of inherited cellular DNA sequences at the level of transcription governs many cancers that are not the result of mutational events (and perhaps even many of those). Thus, transcriptional regulation is of central relevance to problems of differentiation and development. Since viruses are stably integrated nucleic acid sequences in mouse cells and because it is possible to prepare nucleic acid probes and proteins from these viruses, transcriptional regulation can best be approached using RNA-containing tumor viruses. The availability of two distinct

RNA viruses both endogenous to mouse cells, a specific hormonal effect on the virus associated with breast cancer in mice combined with many well characterized cell lines, suggest strongly that results from this system will have widespread biologic significance.

Proposed Course:

(1) Isolation and amplification of mouse mammary tumor viral DNA sequences using recombinant DNA technology. These sequences will be tested for biologic activity to assess the potential risk of high MMTV DNA concentrations. The DNA will be used initially to construct a restriction enzyme map and for binding studies with RNA polymerases and with hormone-receptor complexes.

(2) Continued characterization of virus-related non-MMTV nucleic acid sequences in mammary tumors.

Publications:

Ruscetti, S. K., Scolnick, E. M. and Parks, W. P.: Natural immunity in cats to feline leukemia viral antigens. J. Immunol. 117: 2029-2035, 1976.

Heston, W. E., Smith, B. and Parks, W. P.: Mouse mammary tumor virus in hybrids from strains C57Bl and GR - breeding test of backcross segregants. J. Exp. Med. 144: 1022-1030, 1976.

Levin, R., Ruscetti, S. K., Parks, W. P., and Scolnick, E. M.: Expression of feline type-C virus in normal and tumor tissues of the domestic cat. Int. J. of Cancer 18: 661-671, 1976.

Young, H. A., Shih, T. Y., Scolnick, E. M. and Parks, W. P.: Steroid induction of mouse mammary tumor virus: Effect upon synthesis and degradation of viral RNA. J. Virol. 21: 139-146, 1977.

Scolnick, E. M., Williams, D. and Parks, W. P.: Purification and characterization of the viral RNA of a sarcoma virus isolated from a woolly monkey. Nature 264: 809-811, 1976.

Scolnick, E. M., Williams, D., Maryak, J., Vass, W., Goldberg, R. J. and Parks, W. P.: Type-C particle positive and type-C negative rat cell lines: Characterization of the coding capacity of endogenous sarcoma virus specific RNA. J. Virol. 20: 570-582, 1976.

Troxler, D. H., Parks, W. P., Vass, W. C., and Scolnick, E. M.: Isolation of a fibroblast nonproducer cell line containing the Friend strain of the spleen focus forming virus. Virology 76: 602-615, 1977.

Shih, T. Y., Young, H. A., Parks, W. P., and Scolnick, E. M.: In Vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin: Elongation of chromatin associated RNA by E. coli RNA polymerase. Biochemistry, (in press).

Parks, W. P., and Scolnick, E. M.: In Vitro translation of Harvey murine sarcoma viral ribonucleic acid. J. Virol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04960-02-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Regulation of RNA tumor virus transcription

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M. Chen	Visiting Fellow	LTVG NCI
OTHER:	T. J. Papas	Chemist	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Cellular Transformation Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In an effort to understand the role of the hydrolytic (RNase H) of AMV DNA polymerase activity it was found that this activity could be inhibited to a great extent by cyclic nucleotides while minimum inhibition was observed within the synthetic reaction. ^{32}P-labeled AMV 70s RNA was obtained by limited DNA synthesis in the presence of (α-^{32}P)dATP, TTP, dGTP. Omitting dCTP leads to the synthesis of a short stretch of DNA, seven nucleotides long (AATGAAG) tagged to the 3' end of the tRNA-^{32}P. With labeled AMV 70s RNA p27, p19 and p15 as well as lysoenzyme, all showed binding activity utilizing mitocellulose filters. However, when the binding mixtures at low salt were irradiated with UV and then made 0.5M NaCl, lysoenzyme, p27, p19 showed much stronger binding to AMV RNA than host 28s RNA under similar conditions. Phosphorylation of reverse transcriptase with endogenous virus protein kinase followed by polymerase antibody affinity chromatography showed significant ^{32}P radioactivity coeluting with polymerase activity. A new labeled peptide appeared on SDS gels.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The scope of this investigation is aimed at contributions to the understanding of the mechanism of viral replication with an emphasis on the eventual understanding of cell transformation by RNA tumor viruses.

Methods Employed:

(A) AMV DNA polymerase was prepared in large quantities utilizing pyron-sepharose affinity fractionation. This one-step purification provides homogenous enzyme which is essential for our work. The smaller subunit was prepared from the $\alpha\beta$ complex AMV DNA polymerase by limited exposure to carboxymethylcellulose (CM)-bound trypsin matrix.

(B) Viral 70S RNA is being extracted from AMV as well as RLV under ribonuclease-free conditions. 35S RNA was prepared by heating 70S RNA at 90°C for 3 minutes.

(C) Rabbit globin mRNA was isolated as described by Neinhuis with minor modifications. Total RNA was passed over an oligo(dT)-cellulose column and further purified by sucrose gradient centrifugation. Enzymatic synthesis of cDNA and characterization was carried out according to published procedures.

(D) Sizing of cDNA transcripts was accomplished by electrophoresis in formimide under fully denatured conditions as exponential polyacrylamide gels.

Major Findings:

(A) In an effort to understand the role of hydrolytic activity (RNase H) of the reverse transcriptase, it was discovered that this activity could be inhibited to a great extent with cyclic nucleotides while minimum inhibition was observed within the synthetic reaction. These compounds will be utilized in studying the transcription process.

(B) ^{32}P-labeled AMV 70S RNA was obtained by limited DNA synthesis in the presence of (α-^{32}P)dATP, TTP, DGTP. Omitting dCTP leads to the synthesis of a short stretch of DNA, seven nucleotides long (AATGAAG) tagged to the 3' end of the tRNA-^{32}P. Primer RNA was then obtained by heating tagged 70S RNA. Labeled RNA's were then used in protein-RNA binding assays utilizing nitrocellulose filters. AMV proteins were fractionated by agaro gel column chromatography in the presence of 6M guanidine hydrochloride. Preliminary results indicate a low ionic strength, lysoenzyme,

AMV reverse transcriptase as well as AMV P^{27}, P^{19}, P^{15}, all bind significantly to AMV 70S RNA. Viral major glycoprotein, phosvitin, BSA do not bind to AMV 70S RNA. Similar binding patterns were observed with host 28S ribosomal RNA as with viral 70S RNA when binding assays were done at low salt conditions. At high salt concentrations (0.5M NaCl), viral proteins do not show any significant binding to host 28S ribosomal RNA. Some binding was observed between host 28S RNA and lysoenzyme. With labeled AMV 70S RNA, AMV P^{27}, P^{19} and P^{15} as well as lysoenzyme, all showed binding activity. However, when the binding mixtures (at low salt) were irradiated with UV and then made 0.5 M NaCl, lysoenzyme, P^{27}, P^{19} showed much stronger binding to AMV 70S RNA than AMV P^{15}, although all these viral proteins do not show any binding to the host 28S RNA under similar conditions.

(C) AMV virus showed a low level of endogenous protein kinase activity. Phosphorylation of reverse transcriptase with endogenous virus protein kinase in the presence of (α-^{32}P)-ATP followed by polymerase antibody affinity column chromatography showed significant ^{32}P radioactivity coeluting with polymerase activity. When the ^{32}P radioactivity was pooled, dialyzed and analyzed on SDS PAGE using ^{14}C-labeled enzyme as markers, no ^{32}P radioactivity can be shown in the position of either α or β subunits of reverse transcriptase; however, a new labeled low molecular weight peptide appeared.

Significance to Biomedical Research and the Program of the Institute:

It is clear that the formation, the maintenance, and the expression of provirus are the central features of the life cycle of RNA tumor viruses. Many questions related to these aspects are unsettled. Elucidation of the nature of the enzymes and other factors involved in each step of viral replication and their mechanism of action will be essential to gain an understanding of the process of oncogenesis induced by exogenous and endogenous viruses.

Proposed Course:

(A) To continue the above studies; specifically, to characterize the phosphate acceptor peptide and investigate its possible role in terms of size of cDNA products as well as its fidelity when copying a variety of polynucleotide templates.

(B) To study the mechanism of viral RNA transcription with an emphasis to increase size, yield, and representation of DNA products.

(C) To examine the role of RNase H in the transcription process.

(D) To attempt to reconstitute a ribonucleoprotein complex utilizing the viral genome and purified core proteins. This complex will be further

studied utilizing accumulated information on the structure and function of nucleoprotein complexes within the nucleus of eukaryotic cells.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04962-02-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Oncogenic sequences for feline sarcoma virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Levin	Visiting Fellow	LTVG NCI
OTHER:	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Molecular Virology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A cDNA probe has been prepared to each of the known subgroups of feline leukemia virus and to the sarcoma specific sequences of one of the strains of feline sarcoma virus, the Gardner strain. The cDNA probes homologous to the different feline leukemia viruses indicate that certain lymphomas induced in cats by FeLV (feline leukemia virus) contain RNA and DNA sequences not found in normal feline tissue. The sarcoma specific probe prepared from the Gardner sarcoma virus indicates that its oncogenic sequences are distinct from the oncogenic sequences of another strain of feline sarcoma virus, the Snyder-Theilin strain. The heterogeneity of sarcoma specific sequences in an outbred population is similar to previous findings in a murine inbred population.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The purpose of this project is to develop a probe specific for the oncogenic sequences of feline sarcoma virus. Such probes have been developed for murine viruses in the past. A probe for a naturally occurring feline virus would allow studies of the epidemiology of this oncogenic information in an outbred population.

Methods Employed:

(1) Preparation of stocks of feline sarcoma virus pseudotyped with cloned feline leukemia viruses such that both the FeSV and FeLV in the stock are both clonal isolates.

(2) Preparation of feline leukemia and feline sarcoma virus cDNA probes which are representative of the genome and specific either for the feline leukemia virus or for the feline sarcoma virus. Performed by hydroxiapatite chromatography and cesium electrophoresis.

(3) Preparation of viral RNA by acrylamide gel electrophoresis from feline sarcoma-leukemia virus mixtures.

(4) Preparation of DNA and RNA from feline cells and other cells infected with feline sarcoma virus and shearing of the nucleic acids by Virtis homogenization.

Major Findings:

(1) Pseudotypes have been prepared with a 1:1 ratio of sarcoma virus to helper virus for both the Gardner strain and Snyder-Theilin strain of feline sarcoma virus.

(2) A cDNA probe has been prepared which is specific for the Gardner strain of feline sarcoma virus. These sarc specific sequences for the Gardner sarcoma virus are not detected in the Snyder-Theilin sarcoma virus or in any of the known isolates of feline leukemia virus. This heterogeneity of sarcoma virus specific sequences noted between the Gardner and Snyder-Theilin strain is similar to that noted earlier in murine systems for the sarcoma specific sequences of the Moloney sarcoma virus versus the Abelson transforming virus. The results again indicate that in a given species not a single oncogenic sequence can account for all of the cancer in that particular species.

(3) Additional FeLV sequences have been detected in the DNA of certain lymphomas induced in cats which are not present in the DNA of normal tissues of cats. These tumors with extra DNA sequences are known to be induced by FeLV and these results are consistent with the models that the FeLV induced tumors in cats are due to horizontal infection.

Significance to Biomedical Research and the Program of the Institute:

The issue of the origin and number of oncogenic sequences in a given species of animal is of vital concern in determining the etiology of cancer in any given species. The studies on this outbred population of cats which indicate that the feline sarcoma virus isolates differ in their sarcoma specific sequences complements earlier experiments performed in an inbred mouse population. These studies indicate that more than one oncogenic sequence must be involved in determining different types of tumors in any given population. Therefore, it is important for future experiments in defining the etiology of a given cancer to define which sets of oncogenic sequences and oncogenic genetic information is involved in the cause of the given cancer in order to attempt to either prevent or treat such cancer.

Proposed Course:

Probes will be prepared from pseudotypes now of the Snyder-Theilin strain of feline sarcoma virus in order to perform reciprocal experiments on it in an effort to strengthen the observations on the heterogeneity of sarcoma specific sequences in this outbred population. In addition, the origin of the sarcoma specific sequences of both the Snyder-Theilin and Gardner strains will be determined by ascertaining if the sequences are present in uninfected cat DNA. The expression of these two sets of sarcoma specific sequences will be tested in various naturally occurring feline tumors in order to see the relative frequency with which either of these oncogenic genetic sequences is expressed in a naturally occurring outbred population of feline tumors.

Publications:

Leven, R., Ruscetti, S. K., Parks, W. P. and Scolnick, E. M.: Expression of feline type-C virus in normal and tumor tissues of the domestic cat. Internatl. J. Cancer 18: 661-671, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04963-02-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical studies on the structure and synthesis of mammalian RNA tumor viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Y. Shih	Expert	LTVG NCI
OTHERS:	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI
	W. P. Parks	Head, Viral Immunogenetics Section	LTVG NCI
	H. A. Young	Staff Fellow	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION Molecular Virology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Mammalian sarcoma viruses are recombinants between helper leukemia viruses and endogenous sarcoma viral sequences. The current project is directed toward understanding the organization of the virus genome and delineating the physical recombination map by two dimensional fingerprints of the RNase T1 resistant oligoribonucleotides of genome RNAs. In another aspect, the endogenous RNA tumor viruses are an important factor in determining cancer in many mammalian species. The cellular control mechanisms of expression of the viral sequences have been studied in the *in vitro* systems transcribing the viral genes.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The major objective of this project during the current fiscal year is twofold:

(1) One is to study biochemically the structure and organization of Harvey and Kirsten sarcoma viruses, their physical recombination maps between the endogenous rat sarcoma virus sequences and their respective murine leukemia viruses as a step toward better understanding the function of mammalian sarcoma virus genomes.

(2) Another objective has been to investigate the *in vitro* transcription of viral DNA sequences in isolated cell nuclei and chromatin in order to further understand the mechanism of transcriptional control of tumor virus genes.

Methods Employed:

(1) The structure of mammalian sarcoma virus genomes. A high resolution two dimensional gel electrophoresis method has been employed to fingerprint the viral RNA labeled with ^{32}P following treatment with T1 ribonuclease. The physical organization of the viral genomes has been studied by reference to the 3'-terminal polyA tract of the T1 ribonuclease resistant oligoribonucleotides.

(2) Transcription of RNA tumor virus genes. *In vitro* transcription of viral DNA sequences in the infected cell nuclei and chromatin has been studied by a new technique of tagging the newly synthesized RNA molecules with Hg atom and subsequent purification over sulphydryl affinity column. This is achieved by carrying out *in vitro* RNA synthesis using 5'mercuridine triphosphate as the precursor.

Major Findings:

(1) The structure of mammalian sarcoma virus genomes. Evidence has been obtained which demonstrates the common identical RNA sequences among the Harvey and Kirsten murine sarcoma viruses and the endogenous rat sarcoma viruses, thus further substantiating the notion that sarcoma viruses are recombinants between helper murine leukemia viruses and endogenous rat cell sequences.

(2) Transcription of RNA tumor virus genes. The mercury atom tagging method to study the *in vitro* viral RNA synthesis has uncovered a major artifact hitherto unrecognized in interpretation of *in vitro* transcription studies involving isolated nuclei and chromatin. High fidelity of *in*

vitro transcription of Moloney leukemia virus genes in chronically infected cell nuclei and chromatin has been observed. However, most of the viral sequences in the *in vitro* RNA products appear to be initiated and derived from the chromatin associated RNA, and in a sense they are not truly *de novo in vitro* products. Therefore, the *in vitro* transcripts merely reflect the residual *in vivo* gene activities. This observation casts great doubt on any attempt to interpret the faithful transcription of viral sequences as a demonstration of specific regulation of viral genes in the *in vitro* systems.

Significance to Biomedical Research and the Program of the Institute:

It became increasingly clear that mammalian sarcoma viruses are recombinants between helper leukemia viruses and endogenous host cell sequences. The latter sequences appear to be related to the sarcomagenicity of these viruses. Understanding the organization of the virus genomes and sequencing the sarcomagenic element will facilitate identification and future synthesis of polypeptide probes for searching the elusive transforming proteins responsible for malignant transformation of cells.

The discovery of the major artifact in the *in vitro* RNA synthesis has important general implication for interpretation of any *in vitro* transcription studies as related to problems of gene regulation.

Proposed Course:

Studies on organization of mammalian sarcoma viruses will be further pursued. The sequence of the RNA genome of sarcoma viruses will be attempted by a new method of direct sequencing of the DNA transcript of the genome RNA.

Publications:

Young, H. A., Shih, T. Y., Scolnick, E. M. and Parks. W. P.: Steroid induction of mouse mammary tumor virus: Effect upon synthesis and degradation of viral RNA." J. Virol. 21: 139-146, 1977.

Shih, T. Y., Young, H. A., Parks, W. P. and Scolnick, E. M.: In vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin: Elongation of chromatin associated RNA by *E. coli* RNA polymerase." Biochemistry, in press, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04964-02-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of the molecular mechanism of Friend murine erythroleukemia virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. H. Troxler	SA Surgeon	LTVG NCI
OTHER:	E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI
	W. P. Parks	Head, Viral Immunogenetics Section	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION Molecular Virology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Friend murine erythroleukemia virus contains a replication defective leukemogenic viral component, the spleen focus-forming virus (SFFV), and a replicating helper virus component, the Friend murine lymphatic leukemia virus (F-MuLV). We have for the first time cloned SFFV free of F-MuLV by cell culture techniques, and have confirmed that it is replication defective and causes the rapid erythroleukemia induced by the Friend virus complex. By isolating SFFV free of helper virus in nonproducer cells, we have further been able to characterize the genome of SFFV by molecular hybridization; we have shown that it contains at least 2 discrete portions, one portion homologous to F-MuLV and another portion partially homologous to xenotropic virus.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The objective of this project is to study the mechanism by which the Friend murine erythroleukemia virus causes rapid leukemia in mice. The Friend virus complex consists of 2 discrete viral components: SFFV, which is replication defective but which causes rapid erythroleukemia, and F-MuLV which is a replicating type-C RNA tumor virus which causes lymphatic leukemia. The goal of this project is to study the nature and origin of the nucleotide sequences contained in SFFV as well as to attempt to identify a SFFV-specific protein which causes Friend leukemia. Further, we intend to study the Gross murine leukemia system and radiation induced leukemia system using techniques developed in the above studies.

Methods Employed:

(1) Tissue culture: Virus cloning, cell cloning, XC plaque virus assays, isolation of nonproducer cells containing SFFV.

(2) Animal work: Inoculation of newborn and adult mice and newborn rats with various MuLV to document and characterize their pathogenic potential. Use for spleen focus-formation assays as well.

(3) Preparation of cDNA probes from Friend virus complex utilizing both exogenous and endogenous reverse transcriptase reactions; absorption of nucleotide sequences specific for the replicating helper virus from this cDNA. These methods involving nucleic acid hybridization techniques, hydroxylapatite cycling, cellular and viral RNA extraction, and preparation of 70S viral RNA.

(4) Developing *in vitro* protein synthesis techniques.

Major Findings:

(1) The Friend strain of spleen-focus-forming virus has been cloned in cell culture free of F-MuLV in both mouse and rat fibroblasts. F-MuLV has also been extensively cloned and it causes a lymphatic leukemia in susceptible mice.

(2) DNA probes for F-MuLV and SFFV have been obtained and have been shown to correlate well with disease induced. The SFFV has been characterized by molecular hybridization and like other replication-defective rapidly-transforming murine RNA tumor viruses, it contains nucleotide sequences homologous to a portion of the helper virus genome; in addition, it

contains nucleotide sequences not contained in F-MuLV (SFFV-specific) but partially related to xenotropic MuLV.

(3) The Friend strain of SFFV therefore has been shown to be a recombinant virus which arose by recombination between F-MuLV and endogenous viral sequences partially homologous to xenotropic virus. We are currently investigating the nature, expression and origin of these SFFV-specific sequences.

Significance to Biomedial Research and the Program of the Institute:

The mechanism by which murine leukemia viruses actually cause leukemia is unknown. We have studied the Friend erythroleukemia virus complex to attempt to characterize the viral component which causes rapid leukemia. The results obtained to date suggest that the leukemogenic SFFV arose by recombination between helper virus and sequences endogenously present in mouse DNA and related to xenotropic viruses, and these studies for the first time indicate a direct role for recombination and xenotropic MuLV in leukemogenesis. It is hoped that further elucidation of the natural expression of the SFFV-specific nucleotide sequences as well as characterization of a SFFV-specific protein will allow us to propose a model for leukemic transformation which will have direct and profound implications for human leukemia.

Proposed Course:

The project will continue to study the Friend erythroleukemia virus complex with particular emphasis on, (a) the role of the xenotropic-related sequences in leukemogenicity, (b) the nature of the recombinational event which led to SFFV, (c) the natural expression of the SFFV-specific sequences, and (d) the mechanism of leukemia-induction by F-MuLV and other cloned MuLV. We also intend to attempt to identify a protein or proteins responsible for SFFV-induced leukemia.

Publications:

Troxler, D. H., Parks, W. P., Vass, W. C. and Scolnick, E. M.: Isolation of a fibroblast nonproducer cell line containing the Friend strain of the spleen focus forming virus. Virology 76: 602-615, 1977.

Troxler, D. H., J. K. Boyars, W. P. Parks, and E. M. Scolnick: The Friend strain of spleen focus-forming virus: A recombinant between mouse type-C ecotropic viral sequences and sequences related to xenotropic virus. J. Virol. May, 1977 (in press).

Papers Presented at Meetings:

Troxler, D. H. et al. Isolation of a fibroblast nonproducer cell line containing the Friend strain of spleen focus forming virus. Presented at the Annual Meeting of the Virus Cancer Program, Hershey, Pennsylvania, November, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04965-01-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Replication of and cellular controls on murine oncornaviruses in cell cultures

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. R. Rein	Expert	LTVG NCI
OTHER:	R. H. Bassin	Head, Viral Biochemistry Section	LTVG NCI
	B. I. Gerwin	Chemist	LTVG NCI
	G. Duran-Troise	Visiting Associate	OD DCCP NCI
	B. Barlow	Visiting Fellow	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION

Viral Biochemistry Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this work is to understand the replication cycle of murine leukemia viruses (MuLV's), and the mechanism of Fv-1 restriction, a naturally occurring cellular system which specifically inhibits the replication of certain MuLV's. The studies use a number of cell culture techniques for quantitation of virus replication.

Defective variants of MuLV have been isolated. Biological techniques for the study of these variants have been developed, including a method for rescuing defective MuLV and a plaque assay for quantitating defective MuLV particles. These variants will be used for genetic studies on MuLV replication.

Previous work from this laboratory has shown that tropic MuLV's possess nongenetic determinants which render them sensitive to Fv-1 restriction, and that these determinants can be donated to other viruses by phenotypic mixing. In an extension of this work, the defective variants discussed above were shown to donate tropism to an NB-tropic MuLV, which is normally insensitive to Fv-1 restriction.

Project Description

Objectives:

The goal of this project is to understand the replication cycle of murine leukemia viruses and the mechanisms by which cellular control mechanisms can affect this cycle. The project has focused on two areas of experimentation:

(A) A study of the mechanism of action of the cellular gene, Fv-1, on the replication of murine leukemia viruses (MuLV's).

(B) Studies on replication-defective variants of MuLV.

Methods Employed:

Tissue culture assays for virus infectivity; development and characterization of specialized cell lines producing replication-defective variants of MuLV; analysis of viral interference; virus cloning techniques; complementation assay for defective MuLV.

Major Findings:

(A) A number of specialized reagents for the analysis of the MuLV genome and the mechanism of Fv-1 restriction have been developed. One of these reagents is an isolate of NB-tropic MuLV (i.e., MuLV which is insensitive to Fv-1 restriction) which is unique in that it can overcome viral interference. In addition, a large number of cell clones have been isolated which are infected with MuLV but produce noninfectious virus particles.

These clones producing noninfectious particles have been studied in detail by a variety of techniques, including superinfection studies, molecular hybridization, and transfection. The particles produced by these clones have been analyzed for biological activity, and their proteins have been analyzed by polyacrylamide gel electrophoresis. The results of all of these studies provide strong evidence that these clones are infected with replication-defective variants of MuLV.

(B) These clones do not induce fusion in XC cells. As reported by Hartley and Rowe (1976), cells infected by the amphotropic isolate of MuLV (MuLV-amph) also do not induce fusion in XC cells. However, when clones infected with replication-defective MuLV variants are co-infected with MuLV-amph they do induce XC cell fusion. This appears to represent complementation between MuLV-amph and replication-defective ecotropic MuLV. This complementation has been used to develop a plaque assay for replication-defective

ecotropic MuLV. In turn, results obtained with this assay show that MuLV-amph can rescue defective MuLV from a clone infected with a replication-defective variant of Moloney MuLV. The mechanism of rescue of defective MuLV appears analogous to that of rescue of defective MSV.

(C) (1) The NB-tropic MuLV isolate described in (A) above was used to superinfect clones infected with replication-defective variants of N- or of B-tropic MuLV. In the case of some of the clones, the infectious MuLV which was then produced was found to have acquired N- or B-tropism by phenotypic mixing. Thus (a) in agreement with previous work from this laboratory, N- and B-tropic MuLV's specify determinants which are incorporated into virions during virus assembly and confer sensitivity to Fv-1 restriction; (b) NB-tropic MuLV is normally insensitive to Fv-1 restriction because it does not specify these determinants. This result also shows (c) these replication-defective variants do synthesize the determinants of tropism, although they apparently do not synthesize all of the normal products of MuLV infection. This experimental approach is also useful as a specific biological assay for the determinants of tropism.

(2) Some clones producing replication-defective variants of B-tropic MuLV are apparently unable to synthesize the determinants of B-tropism, since they do not donate the B-tropic phenotype to superinfecting NB-tropic MuLV. Thus one defect in these clones appears to involve the determinant of B-tropism. A comparison between the virus-specific proteins produced by these clones, and those produced by clones which do donate the B-tropic phenotype as described in (1), is now under way.

Significance to Biomedical Research and the Program of the Institute:

(A) The development and characterization of new types of reagents in turn allows the design of new experimental approaches to fundamental problems.

(B) Genetic variants are extremely useful in dissecting events in the life cycle of any virus, and in deducing the functions of individual virus-specific products. The clones under study appear to represent a new type of genetic variant of MuLV. The novel plaque assay described above should greatly facilitate quantitative biological studies on these variants.

(C) The Fv-1 restriction system is an example of a highly precise, naturally occurring intracellular control mechanism, in which a cellular substance specifically interacts with some nongenetic component of an MuLV particle and blocks infection by the virus. An understanding of the mechanism of Fv-1 restriction should thus provide new insights into the events which occur during the MuLV replication cycle, the functions of specific virus components in infection, and cellular control mechanisms which can interfere with infection. Once these viral functions and

cellular control mechanisms are understood, they may suggest ways of blocking infections by other RNA tumor viruses in vivo.

Proposed Course:

(A) The reagents will be used as discussed below.

(B) The biological properties of a number of variants will be analyzed in detail. Their biochemical composition will also be studied. These comparative investigations should contribute to our understanding of basic mechanisms in the biology of RNA tumor viruses. In turn, a knowledge of these mechanisms can be expected to lead to new approaches to the inhibition of viral and/or other tumorigenic processes in vivo.

(C) Using the variants described above, studies will be continued to determine the chemical nature of the determinants of N- or B-tropism. In light of recent evidence that Fv-1 restriction blocks integration of proviral DNA in restrictive cells, it seems likely that these determinants are viral proteins which participate in the synthesis or integration of proviral DNA. Thus the identification of these determinants will help us to understand basic mechanisms of virus replication, as well as the mechanism of Fv-1 restriction.

Publications:

Rein, A., Kashmiri, S. V. S., Bassin, R. H., Gerwin, B. I. and Duran-Troise, G.: Studies on the mechanism of Fv-1 restriction: Properties of determinants specifying viral tropism. In Animal Virology, D. Baltimore, A. S. Huang, and C. F. Fox, (eds.) ICN-UCLA Symposia on Molecular and Cellular Biology IV, 269-282, 1976.

Duran-Troise, G., Bassin, R. H., Rein, A. and Gerwin, B. I.: Loss of Fv-1 restriction in BALB/3T3 cells following infection with a single N-tropic murine leukemia virus particle. Cell 10: 479-488, 1977.

Kashmiri, S. V. S., Rein, A., Bassin, R. H., Gerwin, B.I. and Gisselbrecht, S.: Donation of N- or B-tropic phenotype to NB-tropic MuLV during mixed infections. J. Virology (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04968-01-LTVG

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

The molecular studies on the mechanism of transformation of RNA-containing tumor viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. Howard Young	Staff Fellow	LTVG NCI
OTHER:	Dr. E. M. Scolnick	Chief, Lab. of Tumor Virus Genetics	LTVG NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION

Molecular Virology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Previous work from this laboratory has demonstrated that the Harvey and Kirsten sarcoma viruses arose through the recombination of a mouse leukemia virus and endogenous rat genetic information. It has been the aim of this investigation to determine what percentage of the Harvey and Kirsten sarcoma viral information is represented as rat information vs mouse genetic information and where each set of genetic material is located in the viral genome. This information will allow a greater understanding of the mechanism of formation of these sarcoma viruses and how they transform cells.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The approach utilized in this investigation has been to synthesize cDNA which is specific for certain areas of the viral genome. Using purified viral RNA and exogenously added RNA dependent DNA polymerase we have been able to synthesize a cDNA which represents approximately the first 100 nucleotides of the viral genome from both the parent Kirsten murine leukemia virus and the Kirsten sarcoma virus. By the use of RNA-DNA hybridization, it has been possible to determine the amount of murine and rat genetic information present in this unique segment of cDNA.

Methods Employed:

(1) Purification of viral RNA by sucrose density sedimentation of sodiumlauryl sulfate disrupted virus.

(2) Synthesis of cDNA in an *in vitro* reaction using purified viral RNA, exogenous RNA-dependent DNA polymerase and ^{3}H-labeled dCTP as the radiolabelled marker.

(3) Molecular hybridization - cDNA-RNA hybrids were analyzed using S_1 nuclease.

(4) Sizing of cDNA - cDNA was sized through the use of SDS-polyacrylamide gel electrophoresis. Autoradiography was performed on 10% acrylamide slab gels with appropriate markers to determine the approximate size class of the cDNA.

Major Findings:

(1) Through the use of 70S viral RNA and exogenous RNA-dependent DNA polymerase we have succeeded in synthesizing cDNA from Kirsten murine leukemia virus and Kirsten sarcoma virus of approximately 100 to 125 nucleotides in length. This RNA, primed by the endogenous tRNA represents the very 5' end of the viral RNA.

(2) Molecular hybridization to RNA from numerous cell lines expressing either the Kirsten leukemia or Kirsten sarcoma virus information has demonstrated that some, but not all, of the 5' end of Kirsten leukemia virus is part of the Kirsten sarcoma virus.

(3) We have also shown that rat specific genetic material is present at the 5' end of the Kirsten sarcoma virus suggesting that the formation of

the Kirsten sarcoma virus may have arisen through a recombinational event occurring at the 5' end of the Kirsten murine leukemia virus.

Significance to Biomedical Research and the Program of the Institute:

By determining how the Kirsten and Harvey sarcoma viruses may have been formed through genetic recombination it will be possible to study more directly the rat genetic information which is responsible for cellular transformation. Since this information is endogenous in the rat, i.e. integrated into the total rat genetic information, a fuller understanding of how this information is regulated in the rat will serve as an important model for similar genetic information which may be responsible for human oncogenesis.

Proposed Course:

Current studies will allow us to determine if the mouse sequences present in Kirsten leukemia virus are found elsewhere in the viral genome in addition to the 5' end. In addition cDNA specific for the 3' end of the viral genome will be synthesized to determine the origin of these sequences. Furthermore, by obtaining poly A fragments of the viral genome it may be possible to map the location of the individual viral genes.

Publications:

Young, H. A., Shih, T. Y., Scolnick, E. M. and Parks, W. P.: Steroid induction of mouse mammary tumor virus: Effect upon synthesis and degradation of viral RNA. J. Virol. 21: 139-146, 1977.

Shih, T. Y., Young, H. A., Parks, W. P. and Scolnick, E. M.: In Vitro transcription of Moloney leukemia virus genes in infected cell nuclei and chromatin: Elongation of chromatin associated RNA by E. coli RNA polymerase. Biochemistry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04970-01-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemistry of cellular transformation by avian sarcoma viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. P. Bader	Head, Cell. Transformation Section	LTVG, NCI
OTHER:	R. Silva	NIH Post-doctoral Fellow	LTVG, NCI
	T. Okazaki	Visiting Fellow	LTVG, NCI
	W. Iglewski	Guest Worker	LTVG, NCI
	D. Marciani	Expert	LTVG, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Cellular Transformation Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Malignant transformation by Rous sarcoma virus is induced by a virus-coded protein which plays no role in virus reproduction. Immunoselection techniques have identified a series of virus-specific proteins in a variety of avian and mammalian cells transformed by two strains of Rous sarcoma virus. Some of these proteins are viral structural proteins, or precursors of virion proteins. Several other unidentified proteins are candidates for the transforming protein, and more sensitive techniques are being applied to distinguish virion proteins from the transforming protein and its possible precursors. The function of the transforming protein has been examined using a virus mutant inducing temperature-dependent transformation. The protein apparently has an enzymatic modifying function, probably an involvement in glycosylation of proteins or lipids. This modifying action has a direct effect on cellular membranes resulting in an increased uptake of sodium ions. The unequivocal identification of a virus-coded transforming protein and resolution of its function appears attainable.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To identify the virus-coded protein responsible for the malignant transformation of cells by Rous sarcoma virus; to determine the biochemical function of this protein; to determine the primary physiological effects resulting from the functioning of this protein; to describe the sequence of metabolic changes which result in the altered metabolic profile characteristic of malignant cells; to distinguish metabolic changes necessary to the maintenance of the malignant state from those which are irrelevant.

Methods Employed:

(A) Transformation of cells in culture by avian and murine sarcoma viruses, selection of mammalian cells transformed by avian viruses, morphological resolution of transformed cells by microscopy, resolution of cellular organelles by supravital staining.

(B) Isolation of cellular organelles, including microsomes, cell surface membranes, Golgi membranes, and nuclei; determination of cell diameters, volumes and densities.

(C) Chemical determinations of protein, acid and alkaline phosphatases.

(D) Complement fixation, immunoprecipitation, immuno-affinity chromatography.

(E) Dextran and sucrose density gradients, polyacrylamide gel electrophoresis, autoradiography, fluorography.

(F) Uptake of radioactive molecules into cells, incorporation of radioactive precursors into macromolecules, binding of radioactive molecules to specific cell-surface sites.

Major Findings:

Attempts have been made to identify the virus-coded protein responsible for the malignant transformation induced by Rous sarcoma virus, and to determine the function of this protein. Direct analyses of the proteins of entire cells, of fixed cell surface membranes, of unfixed cell surface membrane vesicles, and of microsomes, revealed substantial decreases in two proteins, m.w. 210,000 (LETS protein) and m.w. 40,000 (actin), as reported by others, and loss of a third protein, m.w. 70,000, previously

unreported. The decreases in these proteins were concluded to be late events in virus transformation, occurring long after other morphological and biochemical changes characteristic of transformation had occurred. No obvious proteins suggestive of virus gene products were apparent in these preparations.

Immunoselection techniques were used in attempts to separate virus-specific proteins from cellular proteins. A variety of mammalian cells derived from rat, hamster, horse, and rabbit tissues were infected with two strains (Bryan "high titer" strain and Schmidt-Ruppin strain) of Rous sarcoma virus. Transformed colonies were isolated and propagated, and cell lines were established. These cell lines were used as antigens to induce antibodies in rabbits. Induction of complement-fixing antibodies against viral structural proteins was used as an indicator of successful antibody induction. Positive antisera were used in immunoprecipitation and immunoaffinity chromatography for the binding of proteins labeled in cell cultures with radioactive amino acids. Heterologous antigen-antibody complexes, e.g., hamster vs. chicken, or rat vs. hamster, selected less than 20% of the original proteins in immunoprecipitation experiments, and less than 5% in immunoaffinity chromatography experiments. After electrophoresis in polyacrylamide gels, the patterns of bands were distinct from the patterns developing from unselected extracts. Several protein bands with molecular weights characteristic of viral strucutral proteins were identified, as well as a few likely large molecular weight precursors to these structural proteins. A number of other bands possibly are partially processed intermediates between the original precursor and the finished structural proteins. Elimination of several viral structural proteins from consideration was accomplished by preincubating antibody preparations with excessive amounts of unlabeled virion proteins. Residual bands are now being scrutinized in the hopes of unequivocally identifying the virus-coded transformation protein. The recent acquisition of transformed rabbit cell lines allows the hope of greater specificity in the immunological selection of the transforming protein, since the rabbits burden of developing antibodies against the large number of heterologous cellular proteins will be removed.

Determining the function of the virus-coded protein responsible for malignancy has been approached using a mutant of Rous sarcoma virus which induces temperature-dependent transformation. Infected cells at 41° appear nontransformed, but after shifting to 37° a variety of morphological and biochemical changes occur. Many of the biochemical changes have been shown to be late events in transformation, since these changes can be prevented by inhibitors of RNA or protein synthesis. Nonetheless, under the same conditions all of the morphological changes characteristic of transformation occur, including rounding, enlargement, cytoplasmic vacuolization, and loss of orientation. The induced vacuoles, the most distinctive feature of these cells, were shown to be composed largely of water, and were bounded

by membrane. Neutral Red and other unbranched cationic dyes readily entered the vacuoles, and were concentrated there from the medium, while anionic dyes were excluded. Other experiments involving a variety of cations indicated that vacuoles are involved in the cellular uptake of Na^+. In fact, Na^+ is taken up more rapidly by transformed vacuolated cells than by nontransformed cells, while K^+ uptake is similar in transformed and nontransformed cells. The dissociation of Na^+ and K^+ fluxes in these cells was confirmed with the demonstration that there were no differences in Na-K ATPase in cells with increased rates of Na uptake. The development of the vacuole can be prevented by small amounts of deoxyglucose, a sugar which interferes with glycosylation reactions, suggesting that the virus-coded protein may be a glycosylating enzyme or an acceptor for glycosylation. Preliminary experiments revealed no extraordinary glycosylating activity in extracts, or in membrane fractions, of transformed cells. Analysis of membrane glycoproteins is in progress, testing the possibility of identifying a unique glycoprotein in transformed cells.

The possibility that increased intracellular Na^+ levels might be sufficient to release cells from normal constraints and effect malignancy is under investigation. Exposure of nontransformed cells to increasing exogenous Na^+ concentrations resulted in proportional increases in uptake of glucose and amino acids, although no effects were found on rates of macromolecular synthesis, cell growth, or saturation density. While failing to mimic malignant transformation, a hypothesis espoused by Holley and by Pardee has been disproved, i.e., that the increased cellular uptake of metabolites effected at the cell surface membrane of transformed cells is responsible for malignancy. Nonetheless, an already high available intracellular Na^+ concentration in transformed cells, indicated above, was supported by the failure of graded exogenous Na^+ concentrations to affect uptake of metabolites in transformed cells. Phosphatases are recognized components of cell membranes. In investigating the nature of membrane changes occurring during transformation, an increase in acid phosphatase, and the disappearance of alkaline phosphates were found - two more biochemical changes to add to the catalogue of differences between transformed and nontransformed cells.

Significance to Biomedical Research and the Program of the Institute:

The identification of a single protein responsible for malignant transformation, and resolution of that protein's function, would be major advances in our understanding of the nature of cancer cells. Recognition of a primary physiological imposition on the cell which converts that cell to a malignant form would allow a description of the entire sequence of metabolic events which culminate in malignancy. Such studies could lead to a determination of metabolic events necessary to the maintenance of the malignant state, but not to normal metabolism, and the possibility of therapeutic intervention in such metabolism.

Proposed Course:

Use of more specific selective techniques to separate the virus-coded transforming protein from the relatively vast amounts of cellular and viral structural proteins. Resolution of primary and secondary metabolic changes during the development of transformation in a temperature-conditional system. To examine the intracellular role of monovalent cations in the regulation of RNA and protein synthesis, and the possible role of these cations in induction and maintenance of the malignant state in the Rous sarcoma system.

Publications:

Bader, J. P., Lew, M. A. and Brown, N. R.: Uptake of exogenous metabolites by virus-transformed cells: Changes induced by temperature and pH. Arch. Biochem. Biophys. 175: 196-208, 1976.

Bader, J. P.: Sodium: A regulator of glucose uptake in virus-transformed and nontransformed cells. J. Cell. Physiol. 89: 677-682, 1976.

Bader, J. P. and Brown, N. R.: Cytoplasmic vacuoles of Rous transformed cells are organelles involved in cation uptake. Int. J. Cancer (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04971-01-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Biochemical properties of neoplastic cell membranes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. J. Marciani	Expert	LTVG, NCI
OTHER:	J. P. Bader	Head, Cell. Transformation Section	LTVG, NCI
	R. J. Goldberg	Microbiologist	OADVO, NCI
	E. B. Thompson	Medical Director	LB, NCI

COOPERATING UNITS (if any)
Laboratory of Biochemistry, DCBD

LAB/BRANCH: Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION: Cellular Transformation Section

INSTITUTE AND LOCATION: Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The modification of membrane components, mainly proteins that occur in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus are studied in order to ascertain the role of such modification in alterations of cell properties that are expressed at or mediated by the cell surface. Analysis of the role of specific cell surface polypeptide present in somatic cell hybrids in the expression of some normal cellular properties. Cell membranes are isolated and their composition determined by electrophoretic and chromatographic procedures. Membrane surface properties are investigated by the use of lectins and labeling with surface-selective techniques. Relations between different polypeptides in somatic cell hybrids are investigated by peptide mapping.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The study of membrane components, mainly proteins, that can be modulated in cells transformed by a temperature-sensitive mutant of Rous sarcoma virus, or in somatic cell hybrids between malignant cell mouse LB82 and rat hepatoma HTC-1. The study of the role of these proteins in properties of the cell that are mediated by, or expressed at, the cell surface.

Methods Employed:

Isolation of cell membranes in an aqueous two-phase system, or by induction of cell vesiculization and separation in density gradients. Analysis of the membrane composition by electrophoretic and chromatographic techniques. Study of the cell surface topography by use of enzymic labeling with ^{3}H or ^{125}I combined with electrophoresis. Isolation of polypeptides expressed in cell membranes of somatic cell hybrids and their parental cell lines for comparison by peptide mapping, in order to determine their control of expression.

Major Findings:

(A) Electrophoretic analysis of cell membranes from chick embryo cells infected with Rous sarcoma virus (RSV) and with a mutant (Ta) of RSV, have revealed: (1) That in transformed cells the 250K mol. wt. region, which corresponds to the "large external transformation sensitive protein" or LETS, shows apparent microheterogeneity, perhaps due to differences in glycosylation. (2) Cells infected with Rous sarcoma virus showed a new polypeptide in their cell membranes, the same that seems to be present in cells infected by RSV-BH-Ta but in different proportions. (3) Receptors for Lens culinaris lectin were isolated by affinity chromatography. The major receptor in normal cell membranes is the LETS protein, whereas in cells transformed by RSV or its mutant is a glycoprotein with electrophoretic mobility similar to gp80 or the major glycoprotein from the virus.

(B) Binding of ^{3}H concanavalin A to RSV-BH-Ta infected cells at different times of temperature shift from 41° to 37°C resulted in a decrease of binding sites at the cell surface, a process that occurred without an appreciable lag period. The increase in binding sites in cells shifted from 37°C to 41°C occurred after a lag period of 12 hours and was completed in 48 hours. Examination of the kinetics of changes in binding sites as a function of shift time suggests that the products responsible for the increased number at 41°C are unstable at 37°C. The lag period observed in

cells shifted up indicates an absence in the cell at 37°C of the required products, and that in order to observe the changes, DNA or protein synthesis is required.

Significance to Biomedical Research and the Program of the Institute:

The fact that the sociology of cells is mediated through their cell surfaces makes the study of cell membranes important for understanding the lack of contact inhibition, the changes in permeability, and the capacity for invading normal tissue that are characteristic of neoplastic cells.

Proposed Course:

We are planning to study the change in glycoproteins at the plasma membrane level as a function of temperature shift in cells infected with RSV-BH-Ta. These studies will involve isolation of glycoproteins by electrophoresis and characterization of the glycosylated moiety after extensive digestion with pronase, by gel filtration and electrophoresis. Isolation of newly expressed polypeptide(s), and characterization by comparison with structural proteins from the virus and with similar polypeptides isolated from cells infected by leukosis virus.

Using somatic cell hybrids we intend to follow the changes in cell membrane as a function of chromosome loss and try to correlate changes with other characteristics, such as growth properties.

Publications:

Marciani, D. J. and Okazaki, T.: Interaction of Con A with chick embryo fibroblast transformed by Rous sarcoma virus. Biochim. Biophys. Acta 455: 849-864, 1976.

Papas, T. S., Marciani, D. J., Samuel, K. and Chirikjian, J. G.: Mechanism of release of active α subunit from dimeric αβ avian myeloblastosis virus DNA polymerase. J. Virol. 18: 904-910, 1976.

Marciani, D. J., Lyons, L. B. and Thompson, E. B.: Characteristics of cell membranes from somatic cell hybrids between rat hepatoma and mouse L-cells. Cancer Res. 36: 2937-2944, 1976.

Papas, T. S., Pry, T. W. and Marciani, D. J.: Inactivation of avian myeloblastosis virus DNA polymerase by specific binding of pyridoxal 5'-phosphate to triphosphate binding site. J. Biol. Chem. 253: 1426-1430, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04972-01-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on cellular control of infection with RNA tumor viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	B. M. Barlow	Visiting Fellow	LTVG NCI
OTHER:	R. H. Bassin	Head, Viral Biochemistry Section	LTVG NCI
	B. I. Gerwin	Chemist	LTVG NCI
	A. R. Rein	Expert	LTVG NCI
	G. Duran-Troise	Visiting Associate	OD DCCP NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION
Viral Biochemistry Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fv-1 restriction in NIH and C_3H (Fv-1^n) cell lines were studied using tropic pseudotypes of MSV. Evidence for two levels of restriction was found in preliminary experiments. One type of restriction appears to be similar to that previously reported in Balb/3T3 (Fv-1^b) cells, while an additional restriction, which may or may not be related to the presence of the Fv-1^n gene, may also be present.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

This project is concerned with the mechanism of Fv-1 dependent intracellular restriction of N- and B-tropic murine leukemia and sarcoma viruses in mouse cells. This phenomenon, which is known to be controlled by a single autosomal gene (Fv-1), is of special interest, since an understanding of how restriction operates may be fundamental for studies on the cellular control of RNA tumor virus expression and on the nature of virus replication and cell transformation in general.

Methods Employed:

The studies involve a variety of cell culture techniques for the quantitation of virus replication and cell transformation. In addition to routine procedures Fv-1 related and other restriction phenomena were analyzed by quantitative dose-response assays, some of which were designed in this laboratory. Various S+L- mouse lines also have been developed for quantitation of different viral functions associated with MuLV and MSV infection.

Major Findings:

Recent studies have emphasized the restriction of B-tropic MuLV stocks in N type cells. These studies have involved the use of tropic pseudotypes of MSV to measure Fv-1 restriction in C_3H and NIH/3T3 cell lines. It is known that the restriction of tropic MuLV is quantitatively different in N cells than in B cells. Our initial experiments indicate that this may be the result of two levels of restriction present in N cells. One restriction is virtually identical to that observed in Balb/3T3 cells, and is probably related to the presence of the $Fv\text{-}1^n$ allele. The second level of restriction, which is superimposed upon the first, may or may not be related to Fv-1 and appears to be affected by the presence of MSV.

Significance to Biomedical Research and the Program of the Institute:

The Fv-1 restriction system appears to represent a model for intracellular control of RNA tumor virus infection. Study of this restriction in a variety of nonpermissive virus-cell systems may give us a better understanding of RNA tumor virus replication and infection. Further, techniques whereby cellular restriction mechanisms can be experimentally modified *in vitro* may be developed using additional experimental data obtained from these systems. Ultimately such knowledge may be applied to *in vivo* experiments, for the potential control or modification of RNA tumor virus infection.

Proposed Course:

These experiments will be enlarged so that Fv-1 restriction in N type cells can be better understood, following to a large extent other previous studies using B type cells. (See Project No. Z01-CP-04848-05-LTVG). In addition experiments are being designed to study the mechanism of a putative second cellular restriction mechanism in Fv-1^n cells.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04991-06-LTVG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Investigations of mammary tumors for viruses and virus-cell interaction studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: H. C. Chopra Research Microbiologist LTVG, NCI

COOPERATING UNITS (if any)
G. Vlahakis, Lab. of Molecular Biology, DCBD, NCI
R. Knazek, Lab. of Pathophysiology, DCBD, NCI
M. Lippman, Lab. of Clinical Medicine, DCT, NCI

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Tumor Virus Genetics

SECTION

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The role of mouse mammary tumor virus (MTV) in the transformation of mammary gland cells was investigated both *in vivo* and *in vitro*. The mammary epithelial cells from preneoplastic mammary glands were cultured, and virus-cell interaction was investigated by light and electron microscopic techniques. Cell lines from various stages of neoplastic conditions were established. By virtue of the fact that the MTV type B as well as its precursor type A particles were recognizable only in the primary cell cultures and they disappeared in the later stages, hormone manipulation is being undertaken to reactivate the virus particles in the established cultures which may reveal the role of MTV in the transformation of mammary gland cells. Electron microscopic studies of human breast tumors is being continued to detect virus-related entities, e.g., "small particles" which will be isolated for biochemical analysis.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

(A) Study of preneoplastic mouse mammary glands to investigate transformation and MTV virus-cell interaction.

(B) Electron microscopic study of human breast carcinomas for virus or virus-related particles.

(C) Collaborative electron microscopic investigations to study viral etiology of cancer.

Methods Employed:

The mammary glands, normal as well as neoplastic, from various strains of mice were cultured in tissue culture media and were examined regularly by light and electron microscopy to investigate morphological transformation. Electron microscopic techniques were also applied to study virus-cell interaction in the mouse mammary tumor cells. Similarly human breast biopsies procured through the Viral Oncology Resources and Services were processed for electron microscopy to detect virus-related "small particles" which will be cultured for biochemical analysis. The electron micrographs were interpreted and evaluated for publications.

Major Findings:

(A) The role of mouse mammary tumor virus (MTV) in the transformation of mammary gland cells continues to be investigated. In vivo transformation studies were conducted previously by light and electron microscopy in the preneoplastic nodules and plaques of C3HfB and DD respectively, and in the mammary tumors of GR and C_3H^{Avy} strains of mice. As reported previously, the preneoplastic conditions for the mammary tumors, i.e., plaques and nodules, demonstrated earlier stages of MTV development but its role and interaction with mammary gland cells remained inconclusive. During this reporting period these investigations were extended to in vitro transformation studies of mammary gland cells. The cell cultures of preneoplastic mammary glands (plaques and nodules) as well as the fully developed mammary tumors were established. Normal mammary glands were also cultured. The cell cultures were systematically examined, both by light and electron microscopy at various stages to follow up the cell transformation and MTV-virus cell interaction. The most significant observations were the epithelial cell growth of the preneoplastic nodules which became much more proliferative than those from the mammary tumors. The cell cultures from the latter consisted more of the fibroblastic cells. No significant morphological differences between the preneoplastic cells and the mammary

tumor cells were recognizable. The role of MTV in the cultured cells of mammary tumors, from MTV positive strain C_3H^{Avy} and from MTV negative but NIV positive C_3HfB were investigated. Even though some morphological differences in various types of mammary cell cultures were observed the role of MTV was difficult to establish by virtue of the fact that the MTV type B particles as well as their precursor type A particles were recognizable only in the primary cultures and they were not detectable after the second passage. This may be attributed to the effect of the lack of hormones in the cell cultures which are prevalent factors in in vivo conditions. Both MTV positive (C_3H^{Avy}) and MTV negative but NIV positive (C_3HfB) cell cultures are well established and being regularly passaged for further hormone manipulation studies to reactivate the viral agents.

(B) Electron microscopic search for virus or virus-related morphological entities in human breast cancer is being continued. The human breast biopsies as well as their cell cultures are being carefully monitored by electron microscopy for virus particles, especially for virus-related cellular components. Since no definite virus particles were identified in the biopsies, the emphasis is being focused on biochemical entities to be fractionated from breast carcinomas. Electron microscopic search of the breast biopsies has demonstrated a characteristic type of particle, "small particle", which is being investigated to establish their viral related entity.

(C) During the past reporting period collaborative electron microscopic support to various research projects of viral oncology was provided. Electron microscopic characterization of virus particles in mouse myeloma tumor was conducted and a manuscript is being finalized for publication. Ultrastructural investigations of a rat lymphoblastic leukemia cell culture was completed and a manuscript was submitted for publication. Human breast cancer cell line (MCF-7) was investigated to describe in vitro formation of solid human mammary carcinoma. I have also participated in the Breast Cancer Virus Workshop organized by the Virus Cancer Program of NCI to report virus-like particles in human milk resembling the Mason-Pfizer virus isolated from the monkey breast tumor.

Significance to Biomedical Research and the Program of the Institute:

The role of viruses in causing cancer in animal systems has contributed significantly in their role to cause cancer. However, the role of virus in human cancer as yet remains to be established and our present investigations shall contribute to understand in general the virus-cell interaction in the animal system and specifically to reveal the etiological role of viruses in human cancer. Our investigations should significantly contribute to the understanding of the role of mouse mammary tumor virus in transformation of preneoplastic mammary gland cells. Similarly electron microscopic

search for virus or virus-related morphological entities in human breast cancer will contribute significantly to the program of the National Cancer Institute.

Proposed Course:

It is planned to extend present studies to biochemical approaches to identify virus-related entities in human breast cancer. The mechanism of virus-cell interaction in the mouse mammary tumors will be investigated by hormone manipulation of the established mammary cell cultures. These objectives will be achieved by undertaking collaborative studies with other investigators.

Publications:

Knazek, R., Lippman, M. E. and Chopra, H. C.: Formation of solid human mammary carcinoma in vitro. J. Natl. Cancer Inst. 58: 419-422, 1977.

Porter, R. P., Van Uiter, G. and Chopra, H. C.: The in vitro and oncogenic characteristics of a rat lymphoblastic leukemia cell culture. In Vitro (in press).

d. Laboratory of Viral Carcinogenesis
July 1, 1976 to September 30, 1977

The Laboratory of Viral Carcinogenesis (1) plans and conducts research on virus-host relationships in viral-induced cancers with emphasis on the detection and characterization of oncogenic viruses and the mode of viral transmission in animals and man; (2) studies the interactions of viral and cellular genes; (3) studies host immune mechanisms related to the control of virus-induced cancers; and (4) conducts investigations on the molecular processes of viral carcinogenesis.

Concerted intramural and collaborative efforts combining diverse and interdisciplinary methods have yielded new insights and approaches. Interactions of cell biology, virology, and molecular methods have allowed further dissections of the central problem which is the understanding of oncornavirus mediated mechanisms of cell transformation and its potential control. Specific objectives included the isolation of murine sarcoma virus specific nucleotide sequences and the determination of their properties, possible origins, and evolution. The translational products of the MSV genome were to be further considered. Because sarcoma viruses are now known to be recombinants, emphasis was given to the isolation of new murine recombinant leukemia type viruses to determine their structure and role in neoplasia. The nature of leukemia-sarcoma virus interactions also led to the specific objective of identifying those antigens which might protect test animals from disease induction under laboratory or natural conditions.

The isolation of complementary DNA's specific for the various segments of MSV RNA resulted in several molecular "probes" representative of such nucleotide sequences as found only in MSV: "sarc", or those sequences shared with MSV and MuLV "common", and those found only in MuLV or "leuk". The "sarc" sequences were found in normal mouse DNA at about one copy per haploid genome. The "common" sequences were found in multiple copies in mouse cells. Related species were examined for presence of "sarc" and "common" sequences to determine their evolutionary relationships. The MSV "sarc" sequences were found as a less related copy and quantitatively less of a copy in other rodent cells. As predicted by taxonomy, this relationship of "sarc" diminished proportionally to the expected species divergence. In contrast the MSV "common" sequences were found only in the mouse and even there the copies were different in that they multiply and were not exact. The "common" sequences already disappeared in related mouse species. Thus two segments of MSV evolved at different rates and were represented in the mouse at different copy numbers. The sarc sequences in mouse cell DNA are highly conserved - in fact as well conserved as the globin gene sequences in mammals. In contrast the common sequences are not conserved and no related sequences can be found in a closely related mouse species - Mus caroli. Hence, the different portions of MSV appear to have a distinct history, supporting the theory of a recent recombination event producing MSV. Further, the sarc sequences may have some important normal cellular function to be so highly conserved.

Because of the presence of "sarc" DNA in normal mouse cells, and because of the case of transformation of mouse cells by a variety of biological and physico-chemical agents, it was asked whether "sarc" was transcribed into RNA in transformed mouse cells. No viral or chemically induced sarcomas of the mouse, other than those transformed by MSV, showed any "sarc" RNA. This ruled out a simple unifying concept of sarcomagenesis.

The role of "sarc" and "common" gene copies in MSV transformed cells was also studied relative to productive and nonproductive infection states. It was interesting that a stable productive infection consistently increased the number of MSV DNA proviral copies in four mammalian systems examined. Increased proviral DNA was also reflected in increased MSV viral RNA.

Additional investigations of MSV on the molecular level have shown that cloned mouse cells which reverted from the MSV transformed state have no expression of MSV specific RNA. These cells are not rescuable for MSV after MuLV infection or induction by halogenated pyrimidines. However, some such revertant clones could back-transform spontaneously and some of these then did express MSV specific RNA indicating that the MSV genome was present during the revertant phase. Accordingly true reversion can occur both by the elimination of the transforming genome or by a complete repression of viral specific functions.

Four classes of mammalian viral RNA subunits were identified according to their homopolyribonucleotide tract composition and the subunits assembled into a proposed genomic model for mammalian retroviruses. The four classes of subunits are different (haploid) if the homopolyribonucleotide sequences are included in the nucleotide composition of the subunits; however, if these sequences are excluded in the nucleotide composition of the subunits, then the question as to whether the viral RNA subunits are haploid or polyploid cannot be answered. The subunits of RNA from mammalian sarcoma viruses were found to be 28S to 30S in contrast to 30S to 40S RNA subunits for leukemia viruses. The 3'-terminal of viral RNA subunits from mammalian leukemia and sarcoma viruses contained poly(A)-rich sequences, and poly(A)-, poly(C)-, and poly(G)-rich sequences were present in the RNA of leukemia and sarcoma viruses; however, poly(U)-rich tracts were absent in the viral RNA.

Hybrid viruses composed of eco- and xenotropic oncornavirus information have been isolated. Collaborative studies indicated that based on peptide maps these are recombinants in the major glycoprotein region which is located near the 3' end of the RNA molecules. This is interesting because the MSV recombined "sarc" sequences are also located in the general region of the genome. To determine whether such recombinant viruses with a very broad host range play an active role in oncogenesis, some cloned variants were inoculated into mice and cats. The recombinant virus was able to induce lymphomas rapidly in several strains of mice but not in cats even after about one year of observation. Other cloned xenotropic murine virus isolates were also examined for recombination and oncogenicity. So far one of these tested was not oncogenic in cats. However, two of these murine xenotropic viruses

were examined for antigenic content and were found to be recombinants in the group specific antigen (gag) region near the 5' end of the molecule. It is of critical importance to relate the recombination event relative to the function of the cell-transforming and tumor-inducing capacities of the recombinant viruses.

The expression of MSV specified proteins was examined in a number of heterologous cells transformed by two variants of the standard MSV. In at least five species of heterologous mammalian cells a precursor polypeptide of the gag region (p60) was expressed in large amounts. One of the MSV variants which also expressed a second larger p70 precursor was also detected. The detection and isolation of MSV specified proteins raises hope for the isolation of the proteins coded by the genes of MSV which maintain the neoplastic state.

All the mammalian and avian type C viruses were found to contain a major structural phosphoprotein. The purified phosphoprotein from each virus specifically binds to the homologous viral RNA genome. Direct and competition binding assays have been developed to study the specificity of such protein-RNA interaction in the different classes of type C viruses. The phosphoprotein from each virus can be subfractionated on the basis of the extent of phosphorylation. Such modification has been shown to modulate the extent but not the specificity of its binding to the viral RNA genome. A subpopulation of the viral structural phosphoprotein, characterized by a low level of phosphorylation, can be specifically recovered from the intact, crosslinked 70S genome of UV-irradiated type C viruses. The close association with the viral genome, the ability of specific interaction with homologous viral RNA and the covalent modification (phosphorylation) suggests a possible regulatory role of the type C viral phosphoprotein in host-virus interaction.

Whereas high leukemic mouse strains were found to carry a highly infectious endogenous ecotropic virus, the ecotropic virus from several low leukemic strains was only poorly infectious. These replication deficient viruses have a very limited host range which excludes rat cells but show a high specific infectivity for mouse cells in which they are expressed as Gross Cell Surface Antigens (GCSA) and cytoplasmic p30 antigen. However, GIX cell surface antigen, other forms of gp70 and gp70 precursors were only barely detectable in infected cells and particle production was exceedingly low. The pathogenicity of these viruses is low as determined by injection into newborn mice of susceptible strains. The replication deficient viruses were converted into a virulent form by passage in certain transformed cells. This conversion was accelerated by mutagens and requires several cycles of infection. Xenotropic virus was activated in the process of conversion. The converted virus is replication competent, has a widened host range which now includes rat cells, is positive in the XC test, and induces GCSA as well as GIX in infected cells. This virus is efficient in activating xenotropic virus in infected cells in vitro and in vivo and is pathogenic in NFS mice. The genetic changes which are associated with these alterations in biologic activity are not detectable by hybridization but are apparent on oligonucleotide fingerprints of the viral RNA genome.

The replication competent ecotropic viruses have been found to be active in in vitro cocarcinogenesis experiments. The relationship between sarcoma virus transformation and the expression of available membrane receptors for several polypeptide ligands were examined. Transformation of cells with either murine or feline sarcoma viruses specifically abolished the binding of epidermal growth factor (EGF), a polypeptide growth factor to its cellular membrane receptors. While the binding of three other polypeptide ligands, fibroblast growth factor (FGF), a multiplication stimulating activity (MSA), and viral glycoprotein, to their membrane receptors were not decreased in murine or feline sarcoma transformed cells.

Epithelioid and fibroblastic clones were isolated from the same source, a normal rat kidney line, and were characterized with respect to their polypeptide ligand receptors and the effect of sarcoma transformation on their expression. The epithelioid clones bound eight times more EGF per cell than the fibroblastic clones. Both cell types upon transformation showed a sharp decrease or a total loss of measurable EGF receptors. The fibroblastic clones, however, bound more MSA than the epithelioid clones, and the MSA binding was independent of murine or feline sarcoma transformation. These substances appear to act as hormones that stimulate certain cells to divide and may have normal physiological functions in development and perhaps during the entire lifetime of an animal. These substances act by binding to specific membrane receptors from which they somehow transmit signals to the nucleus leading to induction of cellular DNA synthesis and other nuclear functions. Cells that lack these specific membrane receptors are unable to be stimulated by exogenously added factors. EGF, for example, appears to have been highly conserved in evolution, as mouse EGF and human EGF crossreact strongly with one another competing equally well for receptor sites on the membranes of various mammalian cells. A substance of this kind has been proposed as a candidate oncogene product. When normal cells were compared to various transformed cells, it was seen that only mouse sarcoma virus transformed cells were altered in the ability to bind exogenous EGF. The MSV transformed cells could not bind EGF while DNA virus transformed cells and most chemically transformed cells bound EGF very well. This does not appear to be the result of virus infection or virus production because non-transforming viruses infecting and growing in these cells did not affect the level of EGF binding. It appears then, in this case that EGF binding is specific for RNA sarcoma virus transformed cells and not for transformed cells in general. Potential uses of this discovery may be to localize specific tumor cells in the body providing a powerful diagnostic and therapeutic tool.

Several independent type C isolates have been obtained from tissues of four species of baboons (Papio cynocephalus, P. hamadryas, P. anubis, and P. papio), from a closely related genus, Theropithecus gelada. These isolates represent a group of closely related primate viruses whose genes have been genetically transmitted in primates for at least 30 million years. To date, endogenous type C viruses have not been isolated from other Old World monkeys and higher apes whose cells are known to contain type C viral genes and occasionally express viral antigens.

A new viral isolate was also obtained from a Southeast Asian primate species, the langur (Presbytis obscurus). This virus, designated PO-1-Lu, is morphologically and biochemically related to the group D retrovirus isolated from rhesus monkeys (Mason-Pfizer monkey virus). Po-1-Lu and MPMV can be distinguished from one another by immunological and nucleic acid hybridization criteria. The isolation of a virus related to MPMV from a second primate species whose natural habitat overlaps that of rhesus monkeys suggests that the group D retroviruses may be endemic to Southeast Asian primates.

Cocultivation of primate tissues with potentially permissive, indicator host cell lines from heterologous species has led to the isolation of recombinant type C viruses containing only a portion of the primate type C viral genome. The new isolates also contain RNA genomes partially homologous to the Kirsten sarcoma genome as well as to mink virogenic information. The ability to obtain such recombinants from nonproductively transformed mink cells suggests that recombination offers one mechanism by which viruses can be obtained from cells which lack the ability to produce infectious, endogenous viruses.

Recent advances in the characterization of the reverse transcriptase reaction have allowed the generation, in vitro, of representative radioactively-labeled DNA copies of the viral genome. Fully representative DNA probes of the baboon, langur, Mason-Pfizer monkey virus, and squirrel monkey virus genomes have been prepared. Data obtained with fully complementary DNA transcripts of the baboon viral genome have confirmed earlier hybridization data. Within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families having more sequence homology than those from distantly related taxons. A comparison of the rates of evolution of viral gene sequences and that of other cellular sequences distinguishes these primates that have evolved in Africa from those that have evolved in Asia. Similar nucleic acid hybridization studies have also shown that the squirrel monkey viruses are endogenous to squirrel monkeys, and are present in multiple copies in the cellular DNA of all tissues and cells of that species.

During the past year several new isolates of murine retroviruses have been obtained from the Southeast Asian murine species Mus caroli and Mus cervicolor. These viruses can be classified into two general groups on the basis of immunological, biochemical, and morphological criteria. The first group is composed of two subgroups of type C viruses isolated from Mus cervicolor. The first subgroup is immunologically related to the woolly monkey type C virus and a type C virus isolated from Mus caroli. This virus has been designated the M. cervicolor C I virus. The second subgroup is immunologically related to but can be distinguished from standard Mus musculus type viruses. This virus has been designated M. cervicolor C II virus. These two subclasses of type C virus can be further distinguished by their host ranges. The C I virus replicates well on tissue culture cell lines derived from either rabbit or cat. The C II virus replicates only on tissue culture cell lines derived from either M. musculus or M. caroli.

Neither class of virus can replicate on M. cervicolor tissue culture cell lines. Sequences related to the viral genomes are present in multiple copies in the cellular genome of M. cervicolor. Less related viral sequences are found in other rodent species including other species of Mus and rats.

The second group of retroviruses exhibits properties which distinguish them from all other types of retroviruses previously described. Isolates have been obtained from M. cervicolor (M432) and M. caroli (M832). Each replicates in tissue culture cell lines derived from M. musculus but not in cell lines derived from the species of origin and were shown by immunologic and nucleic acid hybridization to be highly related but not identical. These viruses contain a 70S RNA genome which can be dissociated into 30S subunits. There are at least six virion associated proteins which are presumed to be encoded by the viral genome. These include the reverse transcriptase, two envelope proteins (gp65 and gp32), and three internal structural proteins (p24, p16, and p12). The major internal protein is p24. The p12 protein is phosphorylated

The properties which distinguish this group of viruses from other retrovirus type include a magnesium-dependent reverse transcriptase with a molecular weight of 70,000 daltons; this differentiates the new isolate from the type C viruses which contain manganese-dependent polymerases and also from MMTV which contains a 100,000 dalton polymerase. The molecular weights of the structural proteins of the M432 isolate also differ from those of murine type B and type C viruses. Moreover, immunologic studies performed with the major internal structural protein (p24) or with the reverse transcriptase have not demonstrated cross-reactions with the homologous proteins of murine endogenous type B and C viruses as well as other retroviral groups. Nucleic acid hybridization data show no detectable sequence homology between the RNA genome of M432 and the genomes of other murine type B and C viruses, while all of the murine type C viruses so far tested (ecotropic, xenotropic, amphotropic) show sequences homology with one another.

Mouse mammary tumor virus (MMTV) proviral sequences were detected in the cellular DNA of mammary tumors and livers of RIII and C3H mice by molecular hybridization with MMTV complementary DNA (cDNA). By means of DNA:DNA reassociation kinetics, the DNA of the mammary tumor cells of these same tumor-bearing mice. Sequences have been isolated of the RNA genomes of the MMTVs from C3H and RIII mice that are transmitted by some mechanism other than via the germ line. These sequences are present as provirus in the DNAs of mammary tumors of C3H, RIII, and GR mice, but are not detected in the DNAs of mammary tumors of C3HfC57BL or BALB/c mice. These sequences are also not detected in the DNA of apparently normal livers of several strains of mice, but are present in the DNA of livers of GR mice. The use of MMTV (^{3}H) labeled complementary DNA (cDNA), prepared in various ways from different MMTV RNAs, demonstrated quantitative differences in MMTV proviral sequences in different mouse strains and between normal and neoplastic tissues of a given mouse.

Mouse mammary tumor viruses (MMTVs) from three different strains of mice have been employed to establish productive infections in feline and mink cell lines. The virions being released by these cells compete completely in a radioimmunoassay for the major virion surface glycoprotein in MMTV (gp52), thus demonstrating that antigenic determinants of gp52 are viral coded. Competitive molecular hybridization studies have shown that the 60-70S RNAs of MMTVs propagated in feline cells contain all the nucleic acid sequences found in 60-70S RNA from MMTV synthesized by murine cells. The virion buoyant densities in sucrose and cesium chloride, virion sedimentation coefficient, divalent cation requirement of the virion DNA polymerase, and morphology of MMTVs synthesized in heterologous cells are similar to those of MMTVs grown in murine cells. Cultures of MMTV-infected feline cells have continuously released between 0.1 and 1.0 μg of virus per 10^7 cells (75 cm^2 flask) per day during the 40 week observation period. No detectable feline or murine type C viruses were produced by these cultures.

Molecular weight determinations of the genomic RNA of MMTV were carried out under aqueous and nonaqueous conditions by polyacrylamide gel electrophoresis and electron microscopy and the results were compared to molecular weights of well characterized avian and E. coli cellular RNA's, tobacco mosaic virus and vesicular stomatitis virus RNA's. The molecular weight of the subunit RNA of MMTV was 2.93-3.1 x 10^6 by gel electrophoresis and 3.09 x 10^6 by electron microscopy, the latter corresponding to a length of 3.44 μm. By extrapolation of semilog plots of the molecular weights of various standard RNA's versus relative electrophoretic mobilities and Ferguson plots, the native RNA of MMTV was 6.45 x 10^6. These data favor the assumption that two subunit molecules comprise the high molecular weight genomic MMTV-RNA.

Two feline chromosomal loci have been studied in detail. The first is Bvr-1 which restricts the production of B-tropic MuLV in cat x mouse hybrids. The restriction occurs late in viral assembly since budding viruses and antigens are apparent on cell surface of restricted cells. Bvr-1 has been mapped to the feline X-chromosome where it is syntenic with Hprt, Gpd and Pgk structural genes for cellular enzymes. The restriction is reversible and does not interfere with gag region polyprotein processing. Bvr-1 gene action is pleiotropic insofar as it restricts the IdU mediated induction of xenotropic MuLV (BALB virus: 2) but not the N-tropic MuLV. Pleiotropic restriction of B-MuLV and X-MuLV but not N-MuLV indicates that the targets of Bvr-1 in B-MuLV and X-MuLV are similar but distinct from those in N-MuLV. The second feline gene is characteristic of the integration site of FeLV. FeLV infected cat mouse hybrids which are segregant for feline chromosomes yield clones which are either positive or negative for FeLV and FeLV-p27 expression. Preliminary evidence suggests that the endogenous FeLV and RD-114 DNA sequences are concordantly inherited in the same hybrids. Discordance of FeLV production and over 20 gene-enzyme markers have been obtained in highly segregant colonies.

A human locus Bevi has been detected in mouse x human and hamster x human hybrid cells. Bevi is required for infection and replication of baboon endogenous virus (M7). The genetic and virological characteristics of Bevi suggest that it may be the chromosomal integration site of M7. Bevi has been mapped to human chromosome 6 where it is syntenic with three gene-enzyme systems, ME, PGM_3, and SOD_2.

Immunological studies showed that a variety of transplantation mouse tumor lines contained murine type C viruses and virus-associated antigens. The type of virus isolated and antigens detected could not invariably be correlated with the original method of tumor induction, but testing of the majority of tumor lines for infectious virus at various levels of in vivo or in vitro passage yielded isolates that were consistent in tissue culture host range for each tumor. In contrast, during the course of in vivo transplantation, some of the lines underwent considerable change in the pattern of virus-associated cell-surface antigens. When the transplanted tumor lines were placed into culture, they showed some alteration in the detectable antigens. Upon retransplantation and passage of the cultured cells into mice, the surface antigens gradually returned to the original in vivo patterns and occasionally acquired additional type C virus-associated antigens not detected in the original tumor line. To test for association of antigens with infectious virus, appropriate tissue culture cell lines were infected with the viruses isolated from the tumors. In these infected indicator cells, some new virus-associated cell-surface and virion envelope antigens were detected, but the complete array of antigens found in the original tumor lines was not acquired.

The radioisotopic footpad assay was found to be sensitive enough for detecting tumor associated antigens in non-virus-induced tumors of mice and rats. Cross reacting tumor associated antigens in methylcholanthrene-induced squamous cell carcinomas of mice were detected by this assay. Human delayed hypersensitivity reactions to living BCg and/or Varidase was adoptively transferred to X-irradiated mice with this assay. The intensity of the footpad reactions to mixtures of test antigens and lymphocytes from human donors correlated with the degree of donor skin test reactivity against the antigen. The anti-tumor immune reaction was adoptively transferred between species by the injection of a mixture of tumor cells and lymphocytes from the tumor-immune or tumor-bearing rats into the foot pad of X-irradiated mice.

Influenza virus-augmented tumor associated antigens were successful for preventing metastasis and regrowth of tumors in mice after excision of tumors. The antitumor footpad reaction became positive in histoincompatible tumor-bearing mice only when the virus-augmented tumor associated antigens were used. The antigenicity of whole intact tumor cells infected with influenza virus was not increased compared to that of non-infected intact tumor cells, while tumor associated antigens isolated from virus-infected tumor cells showed virus augmentation. This suggests that infection of tumor cells with influenza virus stabilizes the tumor associated antigens against chemical degradation.

Cellular immune response detected by ^{3}H-TdR-incorporation in lymphocytes was enhanced significantly by cryosurgery of primary sarcomas. Peak activity was observed 3 days after cryosurgery, whereas marked inhibition of ^{3}H-TdR-incorporation was observed in mice treated with conventional surgery. Cryosurgery elicited significant enhancement of specific immune response to virus-associated antigens on target cells compared to the response to standard surgery or no treatment.

Cell membranes from cultured human melanoma cells infected with vesicular stomatitis virus and UV inactivated gave remarkably stronger skin test reactions compared to membranes from uninfected melanoma cells in 10 human melanoma patients who previously had their tumors surgically removed. The reactivity was specific as shown by the fact that patients with other cancers did not show skin test reactivity.

In cancer vaccine studies in mouse model systems, the dose, route, and timing of immunization with "virus-augmented" tumor cell membrane antigens (i.e., membranes isolated from cells infected with a surface budding virus such as influenza or vesicular stomatitis virus), was determined. Tumor cell membranes not "virus-augmented" induced no tumor immunity.

Methylcholanthrene induced squamous cell carcinomas in mice showed cross reacting transplantation rejection antigens. Previous reports had shown that MCA induced sarcomas did not cross react antigenically. This is the first report that MCA induced carcinomas, rather then sarcomas, do show cross reacting antigens.

Spontaneous neoplastic transformation in vitro is a modified form of foreign body tumorigenesis. This year's results have lead to the conclusion that the concept of "normal" mouse cells in culture may be an invalid one. Instead, they should be viewed as being in the continual process of progression toward spontaneous neoplasia induced by the same causal factor that initiates foreign body solid surface tumorigenesis in vivo: the presentation to the cells of an extensive flat surface on which to attach and grow in the absence of an adequate blood supply. When explants of adult mouse subsutaneous connective tissue were tested for tumorigenicity every two weeks by subcutaneous inoculation of cells either attached to 1x5x10 mm plastic plates or suspended in saline, the plate-attached cells became tumorigenic sooner and in lower dosage.

Positive high titer human sera tested by immunoelectronmicroscopy (IEM) contained natural antibodies to antigens on the envelope of the following type C viruses isolated from primates: gibbon ape leukemia virus (GALV), simian sarcoma virus, baboon endogenous virus, putative human virus isolated from a patient with acute myelogenous leukemia (HL23V) and from human embryonic diploid cells (HEL-12V). Human sera did not react with normal gibbon spleen monolayer cells (GSpl) but showed positive reactions with nonproducing human embryonic diploid cells CIH-1 (V^{-}) which become virus-producing cells CIH-32 (V^{+}) after repeated passages, whereas gibbon sera reacted with GSpl but not with CIH-1 (V^{-}). Human sera strongly reacted

with viral envelope antigen (VEA) of HL-23V common to VEA of GALV, but gibbon sera reacted weakly with it. Human sera cross-reacted with nonproducing human acute lymphocytic leukemia blood cells lines, but gibbon sera did not. Some of the human sera reacted weakly with VEA of feline leukemia virus (FeLV) in the direct IEM, but their reactivity of VEA of GALV was not removed by FeLV-associated surface antigens. Some human sera cross-reacted with VEA of GALV and Rauscher-MuLV. Seventy-nine percent of 76 sera from healthy persons were positive for VEA of GALV with various antibody activity. A peak of antibody level and incidence of positive sera was observed at ages 20 to 50 years, which corresponds inversely to a leukemia incidence with two peaks at ages 1 to 14 years and again over 50 years.

The possibility of oncornavirus control can also be considered from the immunological vantage point in that specific oncornavirus antigens are capable of inducing a neutralizing antibody response. The major glycoprotein of MuLV (gp71) was examined and found to be the major peptide which carries determinants reactive with the neutralizing antibody. A smaller surface antigen, the p15E, was also reactive to a lesser degree. Type, species, and interspecies determinants on the gp71 molecule could all be used for virus neutralization with appropriate antibodies. These findings led to a larger collaborative effort in attempting to use for protection the active immunization with Friend MuLV gp71 in three strains of mice, BALB/c C57BL, and AKR. A summation indicates that in the mouse immunization did result in a type specific response against the F-MuLV gp71 and a concomitant slight protection. However, in the C57/BL strain a derepression of endogenous Gross type virus occurred as determined by type specific anti Gross gp71 antibodies. In the AKR strain immunization was actually deleterious in that mice died of endogenous lymphoma sooner than expected. Accordingly concomitant studies by several laboratories are now actively, and in part successfully, involved in intra- as well as interspecies protection against oncornavirus induced disease by passage antibody mediated immunoprophylaxis.

In addition to their intramural research activities, many of the senior investigators within this Laboratory spent a substantial portion of their time in support of the Virus Cancer Program. These investigators served as Project Officers, Assistant Project Officers, members of coordinating committees and in other supportive capacities. The activities of the Viral Cancer Program and those of this Laboratory are aimed at the common goal of the determination of the viral etiology of human cancer. While the efforts of this Laboratory's scientists on behalf of the VCP have significantly contributed to its progress to date, the broad scientific perspective developed by these investigators in their VCP activities contributed greatly to the direction of the program of this Laboratory. This interaction will continue to underscore the pursuit of the goal of understanding the suspected interaction between tumor viruses and human malignancy. Such a goal will be approached only through the combined efforts of experts in widely differing disciplines within this laboratory and those of collaborating laboratories.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04802-03-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Molecular Studies of Human and Animal Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Schlom	Microbiologist	LVC NCI
OTHER:	G.J. Todaro	Medical Officer, Chief	LVC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Tumor Virus Detection Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The sequences of the RNA genomes of the MMTVs from C3H and RIII mice that are transmitted via a non-germ line mechanism have been isolated. These sequences are present as provirus in the DNAs of mammary tumors of C3H, RIII, and GR mice but are not detected in mammary tumors of C3HfC57BL or BALB/c mice. These sequences are also not detected in the DNA of apparently normal tissues of several strains of mice. Independent polypeptide initiation sites have been demonstrated for the MMTV nonglycosylated proteins (p28, p22, p18, p14, and p10). This is further evidence for the lack of strict coordinate synthesis of these proteins in MMTV-infected cells. MMTVs from three different strains have been employed to establish productive infections in mink and feline cells. These virions contain all the RNA sequences as do murine grown MMTVs. Competitive molecular hybridization studies with MPV 70S RNA demonstrate that MPV and morphologically similar isolates constitute a distinct class of retroviruses. Approximately 20% of the MPV genome is endogenous to rhesus monkeys. This same conserved part of the MPV genome is also endogenous to all other species of old world monkeys tested.

1690

Project Description

Objectives:

These studies are aimed at the use of techniques in molecular biology and immunology to determine a viral involvement in the etiology of mammary carcinoma of experimental animals and humans. The program is divided into several major areas of interest: (a) studies of the mouse mammary tumor viruses (MMTVs), (b) studies of Mason-Pfizer Monkey Virus (MPMV) and related isolates, and (c) biochemical evidence of an oncornavirus involvement in the etiology of mammary carcinoma in other species including humans.

Major Findings:

Mouse mammary tumor virus (MMTV) proviral sequences were detected in the cellular DNA of mammary tumors and livers of RIII and C3H mice by molecular hybridization with radioactively labeled MMTV 60-70S RNA or tritiated MMTV complementary DNA (cDNA). By means of DNA:DNA reassociation kinetics, the DNA of the mammary tumor cells of these two mouse strains were found to contain more MMTV proviral sequences than the DNA of liver cells of these same tumor-bearing mice.

Radioactive 60-70S RNA from the mouse mammary tumor virus (MMTV) produced by the C3H mouse mammary tumor cell line (Mm5mt) hybridized to a greater extent, and at a lower Cot 1/2 value, to the DNA of C3H mammary tumor cells than to the DNA of C3H liver cells. The ^{125}I labeled MMTV(C3H) 60-70S RNA was annealed to a vast excess of DNA from C3H livers and single stranded RNA was eluted from hydroxylapatite and recovered. This "recycled RNA" did not hybridize to the DNA of the apparently normal organs tested from normal or from mammary tumor bearing C3H mice, but hybridized extensively to both the DNA from a C3H mammary tumor cell line and the DNA from spontaneous C3H mammary tumors. This hybridization could be competed out by the addition of unlabeled MMTV 60-70S RNA but was unaffected by the addition of unlabeled 60-70S RNA of C3H type C virus. Similar experiments were conducted with the RIII mouse strain. We have, therefore, isolated the sequences of the RNA genomes of the MMTVs from C3H and RIII mice that are transmitted by some mechanism other than via the germ line. These sequences are present as provirus in the DNAs of mammary tumors of C3H, RIII, and GR mice, but are not detected in the DNAs of mammary tumors of C3HfC57BL or BALB/c mice. These sequences are also not detected in the DNA of apparently normal livers of several strains of mice, but are present in the DNA of livers of GR mice. The use of MMTV (^{3}H) labeled complementary DNA (cDNA), prepared in various ways from different MMTV RNAs, demonstrated quantitative differences in MMTV proviral sequences in different mouse strains and between normal and neoplastic tissues of a given mouse. Results with different (^{3}H)cDNAs were variable, due, in part, to a possible redundancy of certain parts of the MMTV genome as provirus. Thermal analysis of hybrids formed between murine DNAs and MMTV(C3H) (^{3}H)cDNAs, (^{125}I)60-70S RNA, or recycled (^{125}I)RNA, all demonstrated qualitative differences in MMTV proviral sequences in different mouse strains.

Mouse mammary tumor viruses (MMTVs) from three different strains of mice have been employed to establish productive infections in feline and mink cell

lines. The virions being released by these cells compete completely in a radioimmunoassay for the major virion surface glycoprotein in MMTV (gp52), thus demonstrating that antigenic determinants of gp52 are viral coded. Competitive molecular hybridization studies have shown that the 60-70S RNAs of MMTVs propagated in feline cells contain all the nucleic acid sequences found in 60-70S RNA from MMTV synthesized by murine cells. The virion buoyant densities in sucrose and cesium chloride, virion sedimentation coefficient, divalent cation requirement of the virion DNA polymerase, and morphology of MMTVs synthesized in heterologous cells are similar to those of MMTVs grown in murine cells. Cultures of MMTV-infected feline cells have continuously released between 0.1 and 1.0 μg of virus per 10^7 cells (75 cm^2 flask) per day during the 40 week observation period. No detectable feline or murine type C viruses were produced by these cultures.

To distinguish the synthesis of mouse mammary tumor virus (MMTV) structural proteins on a common precursor polypeptide from their independent synthesis on separate ribosome initiation sites, the NaCl hypertonic shock technique has been employed which differentially inhibits polypeptide chain initiation on different classes of messenger RNA in eukaryotic cells. Using this method to inhibit polypeptide initiation in the MMTV-producing C3H mouse cell line Mm5mt/c1, we have selectively inhibited the synthesis of MMTV glycosylated proteins (gp52 and gp36) relative to MMTV nonglycosylated proteins (p28, p22, p18, p14, p10). These results support the concept of independent polypeptide chain initiation sites for the synthesis of these two classes of MMTV proteins. Furthermore, the use of this technique provides evidence for the lack of strict coordinate synthesis of these proteins in an MMTV-infected cell.

Neuraminidase treatment of mouse mammary tumor virus. Rauscher murine leukemia virus and Mason-Pfizer monkey virus resulted in loss of their capacity to inhibit hemagglutination of influenza virus. Hemagglutination-inhibition activity of these RNA tumor viruses could be restored by in vitro resialylation catalyzed by sialyl transferase. The major glycoprotein in the intact envelope of desialylated and, to some extent, native virions could be specifically labeled in vitro with CMP-(^{11}C)sialic acid. These studies further characterize the individual glycoproteins of mouse mammary tumor virus, Rauscher murine leukemia virus, and Mason-Pfizer monkey virus.

The major internal protein p27 (m.w. 27,000 daltons) of the Mason-Pfizer monkey virus (MPMV) was purified by gel filtration and ion-exchange chromatography and then used to develop a radioimmunoassay (RIA). This RIA was specific for MPMV because no immunologic cross-reactivity was observed between p27 or MPMV and 13 different RNA tumor viruses of mammalian and avian origin. However, the p27 of MPMV grown in three different primate cells exhibited identical antigenic cross-reactivity. In addition, significant levels of p27 were found only in MPMV-infected cells. These results indicate that synthesis of p27 is induced after virus infection and that p27 represents a viral-coded protein.

The 60-70S RNA of Mason-Pfizer virus (MPV) was iodinated in vitro and used in both direct and competitive molecular hybridization studies. MPV proviral sequences are present at a frequency of approximately one to two copies per haploid genome in the DNA of experimentally infected human cells. By nucleic

acid competition hybridization, MPV RNA was found to be indistinguishable from the RNA of a virus (X381) isolated from a rhesus mammary gland and from RNA isolated from the cytoplasm of AO cells and HeLa cells, both previously reported to produce MPV-related particles. No homology was observed, however, between MPV RNA and the RNA, or the DNA, from two clones of HeLa cells obtained from the American Type Culture Collection. Hybridization of MPV 60-70S RNA to the DNA of normal tissues of humans and to the DNA of 11 other species revealed that MPV is not an endogenous virus of any of these species. Competition hybridization revealed no detectable sequence homology between the RNA of MPV and the RNAs of simian sarcoma virus, murine mammary tumor virus, murine leukemia virus, BUdR-induced guinea pig virus, or avain myeloblastosis virus. These nucleic acid studies substantiate previous ultrastructural and immunological findings that MPV and morphologically similar isolates constitute a distinct group of oncornavirus.

Iodinated Mason-Pfizer virus (MPV) 60-70S RNA has been used in molecular hybridization experiments to determine the distribution of MPV-specific proviral sequences in the DNAs of primates. Approximately 20% of the MPV genome is present as endogenous provirus in rhesus monkeys. Competitive hybridization experiments showed no homology between MPV 60-70S RNA and the 60-70S RNAs of M7, RD114, and the simian sarcoma virus. No MPV-specific proviral sequences were detected in the DNAs of apparently normal tissues of various species of new world monkeys, apes, and humans. The part of the MPV genome that is endogenous to rhesus is also endogenous to the other species of old world monkeys examined: baboon, African green, and patas. This was determined as a result of the following observations: (a) Cot 1/2 values and final extent of hybridization were the same for all four species; (b) Tm values of MPV 60-70S RNA and DNA of all four species were identical; (c) the removal of MPV sequences endogenous to rhesus tissues by recycling against rhesus DNA, resulted in the loss of any hybridizable MPV RNA to the DNAs of baboon, African green and patas tissues; (d) mixing experiments of rhesus, African green and baboon DNAs resulted in the same kinetics of hybridization as did rhesus DNA alone, when hybridized with MPV 60-70S RNA. These findings demonstrate that sequences that constitute an integral part of the MPV genome are conserved in the DNAs of several different species of old world monkeys.

The inoculation of L2C guinea pig leukemia cells into strain 2 guinea pigs results in the death of the animals within 12 to 15 days. Death is preceded by the simultaneous appearance in the plasma of (a) elevated leukocyte levels, (b) extracellular virus particles, and (c) a particle-associated RNA-directed DNA polymerase. This enzyme activity has a cation preference identical to that of the type B bromodeoxyuridine-induced guinea pig virus, i.e., an Mg^{2+} optimum at 20 mM and no activity using Mn^{2+}. Competitive molecular hybridization studies also revealed that the plasma of leukemic guinea pigs contained approximately 2×10^9 genome equivalents per ml of an RNA that is homologous to the RNA of the bromodeoxyuridine-induced guinea pig virus. Morphological observations indicate that most, but not all, of the extracellular particles observed in leukemia plasma are derived from the intracisternal particles seen in the L2C tumor cells. This presents the possibilities that either two viral populations are present or that the *in vivo* morphogenesis of the type B bromodeoxyuridine-inducible guinea pig virus is markedly different from its *in vitro* morphogenesis.

Significance to Biomedical Research and the Program of the Institute:

These studies are aimed at understanding human and animal mammary cell oncogenesis on a molecular level to determine possible viral cause-effect relationships.

Proposed Course:

To determine the variety and distribution of viral nucleic acid sequences and proteins in murine and primate systems with the ultimate goal of the identification and characterization of viral markers related to human breast cancer.

Publications:

Schlom, J., Colcher, D., Drohan, W. and Schochetman, G.: Nucleic acid and protein studies of Mason-Pfizer virus. In Clemmensen, J., Yohn, D.S. (Eds.): Comparative Leukemia Research. Karger, Basel, 1976, pp. 134-140.

Colcher, D., Drohan, W. and Schlom, J.: Mason-Pfizer virus RNA genome: Relationship to the RNA of morphologically similar isolates and other oncornaviruses. J. Virology 17: 705-712, 1976.

Schochetman, G. and Schlom, J.: Independent polypeptide chain initiation sites for the synthesis of different classes of proteins for an RNA tumor virus: mouse mammary tumor virus. Virology 73: 431-441, 1976.

Schlom, J.: Viruses and breast cancer: A prospective view of approaches applicable to primary prevention and early detection of human breast cancer. Cancer Detection and Prevention (In Press).

Schlom, J., Colcher, D., Drohan, W., Kimball, P., Michalides, R. and Schochetman, G.: Biochemical characterization of mouse mammary tumor viruses and related isolates: Mason-Pfizer virus and the BUdR-induced guinea pig virus. In Hueson, J.C., Mattheiem, W.H. and Rozeneweig, R. (Eds.): Breast Cancer: Trends in Research and Treatment. New York, Raven Press, 1976, pp. 11-51.

Michalides, R., Vlahakis, G. and Schlom, J.: A biochemical approach to the study of the transmission of mouse mammary tumor viruses in mouse strains RIII and C3H. Int. J. Cancer 18: 105-115, 1976.

Schochetman, G. and Schlom, J.: RNA tumor viruses and breast cancer. In St-Arneault, G. and Band, P. (Eds.): Recent Results in Cancer Research. Breast Cancer: A Multidisciplinary Approach. Israel, 1976, Vol. 57, pp. 21-25.

Schloemer, R., Schlom, J., Schochetman, G. Kimball, P. and Wagner, R.: Sialylation of glycoproteins of murine mammary tumor virus, murine leukemia virus, and Mason-Pfizer monkey virus. J. Virology 18: 804-808, 1976.

Michalides, R., Schlom, J., Pearson, J., Perk, K. and Dahlberg, J.: Characterization of the oncornavirus particles in the plasma of guinea pigs with L2C leukemia. J. Virology 18: 1120-1130, 1976.

Schochetman, G., Boehm-Truitt, M. and Schlom, J.: Antigenic analysis of the major structural protein of the Mason-Pfizer monkey virus. J. of Immunology 117: 168-173, 1976.

Kimball, P., Boehm-Truitt, M., Schochetman, G. and Schlom, J.: Characterization of mouse mammary tumor viruses from primary tumor cell cultures. I. Immunological and structural studies. J. Natl. Cancer Inst. 56: 111-117, 1976.

Kimball, P., Michalides, R., Colcher, D. and Schlom, J.: Characterization of mouse mammary tumor viruses from primary tumor cell cultures. II. Biochemical and biophysical studies. J. Natl. Cancer Inst. 56: 119-123, 1976.

Drohan, W., Kettmann, R., Colcher, D. and Schlom, J.: Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in the C3H and RIII mouse strains. J. of Virology 21: 986-995, 1977.

Schlom, J., Schochetman, G., Michalides, R., Kimball, P., Drohan, W. and Colcher, D.: Characterization of the RNA genomes and proteins of the mouse mammary tumor viruses, MPMV and the BUdR-induced guinea pig virus. In Gallo, R.C. (Ed.): Cancer Research: Cell Biology, Molecular Biology and Tumor Virology (In Press).

Schlom, J., Colchér, D., Drohan, W., Kimball, P., Michalides, R., Schochetman, G. and Vlahakis, G.: Propagation and analysis of mouse mammary tumor viruses from different strains. In Schlessinger, D. (Ed.): Microbiology, American Society for Microbiology. Washington, D.C. (In Press).

Schlom, J., Michalides, R., Perk, K., Pearson, J. and Dahlberg, J.: Biochemical properties of the B-type retravirus of guinea pigs and an agent in the plasma of guinea pigs with L2C leukemia. Federation Proceedings (In Press).

Schlom, J., Colcher, D., Drohan, W., Kettmann, R., Michalides, R., Vlahakis, G. and Young, J.: Differences in mouse mammary tumor viruses: relationship to early and late occurring mammary tumors. Cancer (In Press).

Drohan, W., Colcher, D., Schochetman, G. and Schlom, J.: Distribution of Mason-Pfizer virus-specific sequences in the DNA of primates. J. of Virology (In Press).

Howard, D., Colcher, D., Teramoto, Y., Young, J. and Schlom, J.: Characterization of mouse mammary tumor viruses propagated in non-murine cells. Cancer Research (In Press).

Schlom, J., Colcher, D., Drohan, W. and Kettmann, R.: The use of molecular hybridization to track the mode of transmission and distribution of murine mammary tumor viruses: A model for etiologic studies of human breast cancer. In Ito, Y. (Ed.): Progress in Experimental Tumor Research. Volume on "Viruses and Human Cancers" (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04812-09-LVC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

A. Characterization of the high molecular weight genomic RNA in retraviruses
B. Virus-host cell interactions

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	U. I. Heine	Research Microbiologist	LVC NCI
OTHER:	G. J. Todaro	Medical Officer, Chief	LVC NCI
	M. Chirigos	Assistant Laboratory Chief	LRTV NCI
	M. W. Layard	Mathematical Statistician	B NCI

COOPERATING UNITS (if any)

A. S. Dion, Institute for Medical Research, Camden, N.J.
J. Korb, Institute of Molecular Genetics, Prague, Czechoslovakia

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Ultrastructural Studies Section

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. The study was initiated to determine the molecular weights of the genomic RNA's of type B and C retraviruses by electron microscopy and polyacrylamide gel electrophoresis. Visualization of the RNA's was achieved by using a modified Kleinschmidt-technique, permitting optimal extension of the molecules. Standardization of the method was accomplished. The linear density (molecular weight/unit length) calculated as 0.9 x 10^6/μm was found to be constant for different control RNA's comprising a wide range of molecule sizes. The data obtained for the high molecular weight viral RNA's favor the assumption of two subunit molecules constituting the genome of certain retraviruses of type B and C.
B. The study was initiated to characterize by electron microscopic means the morphologic aspects and the virus-host cell relationship of two new virus isolates obtained from M. cervicolor and M. caroli. Both isolates exhibit criteria which permit their classification in the family of retraviridae. A novel association of intracytoplasmic particles of type A, believed to be precursors of the mature virions, with certain cellular organelles was recognized. Evidence is presented of the association of viral precursors with the formation of the centriole.

1697

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To determine the molecular weights of the genomic RNA's of retraviruses of type B and C.

B. To characterize two new virus isolates in respect to their morphology and to their relationship to the host cell.

Methods Employed:

A. Tissue culture techniques, virus propagation and purification, biochemical and electron microscopic methods including the ultrastructural analysis of purified nucleic acids by a modified Kleinschmidt-technique, statistical analysis of different populations of molecules.

B. Transmission electron microscopy, negative staining of purified virus preparations.

Major Findings:

A. Molecular weight determinations of the genomic RNA of the murine mammary tumor virus (MuMTV), a B-type oncornavirus, were carried out under aqueous and nonaqueous conditions by polyacrylamide gel electrophoresis and electron microscopy and the results were compared to molecular weights of well characterized avian and E. coli cellular RNA's, tobacco mosaic virus and vesicular stomatitis virus RNA's. The molecular weight of the subunit RNA of MuMTV was determined to be 2.93-3.1 x 10^6 by gel electrophoresis and 3.09 x 10^6 by electron microscopy, the latter corresponding to a length of 3.44 μm. By extrapolation of semilog plots of the molecular weights of various standard RNA's versus relative electrophoretic mobilities and Ferguson plots, the native high molecular weight RNA of MuMTV was found to be 6.45 x 10^6. These data favor the assumption that two subunit molecules comprise the high molecular weight genomic MuMTV-RNA. The data are in good agreement with previously published studies of the molecular weight and structure of various type C viruses.

For the electron microscopic visualization of the viral genomes the classical Kleinschmidt-technique was modified to permit maximal and optimal spreading under strong denaturating conditions of single-stranded nucleic acid molecules. The linear densities obtained (0.907 x 10^6/μm) by using the refined technique are constant for numerous single-stranded nucleic acids used as controls and they are in good agreement with linear densities obtained by two other spreading methods, the gene 32-protein technique and a non-protein technique developed by Koller et al. The linear densities for different RNA's vary less than 14%; thus, certain easily obtainable, single-stranded nucleic acids, i.e., VSV-RNA, TMV-RNA, r-RNA, can be used for comparison and as standards. In contrast, under moderate denaturing conditions (incomplete denaturation)

higher linear densities were found (up to 1.4 x 10^6/μm) and the degree of extension is different for different nucleic acids, varying up to 40%, therefore excluding the exact calculation of molecular weights under these conditions.

B. The morphology of two new classes of retraviruses isolated from two species of mice [M. cervicolor (virus designated M432), M. caroli] and the relationship of these viruses to their host cells has been investigated. Both isolates exhibit certain characteristics common among virions of type B. The mature particles of 100-120 nm in diameter contain a centrally located nucleoid, a thin intermediate shell and an enveloping membrane derived from the plasma membrane. In negatively stained preparations, M432 virions differ from the caroli virus since only the M432 virions exhibit on their surface spikes of 80-90 A° length and 55 A° width similar to surface knobs of virions of type B. In both classes of viruses, intracytoplasmic particles of type A are precursors of the mature virions. Complete type A particles and incomplete, crescent-shaped type A particles are first observed in close association with the centriole and microtubules, all being embedded in the same matrix. In addition, type A particles, a rare occurrence, are singularly distributed either in the cytoplasm or adjacent to the plasma membrane, where they are released from the cell by budding.

Significance to Biomedical Research and the Program of the Institute:

A. Viruses containing single-stranded RNA as their genome are known to be the causative agents of many tumors in animals, and they are candidates for malignancies in humans. Characterization of the viral genomic RNA is essential for the understanding of viral behavior in regard to nucleic acid replication, the interaction of the genome with host cell DNA, and the events leading to transformation of the infected cells. Standardization of a method used for the calculation of the molecular weights of single-stranded nucleic acids has been achieved by refining the Kleinschmidt technique which permits visualization of nucleic acid molecules in the electron microscope. The results obtained with this method are in excellent agreement with previously established techniques for molecular weight estimation. Using this method and available biochemical techniques, we succeeded in characterizing the genomic RNA contained in the murine mammary tumor virus, a type B virus of the family of retraviridae.

B. Characterization of newly discovered viruses by morphologic aspects may serve as an important aid in their classification, and knowledge in respect to the interaction of viruses with their host cells can be expanded by morphologic studies of the infected cells thus deepening the understanding between virus and host.

Proposed Course:

A. During recent years, our knowledge of the genomic RNA of oncornaviruses has been greatly expanded. For many viruses of such kind data characterizing

molecular weight and configuration of the genome have been computed. The results obtained are confirmed in different laboratories.

In contrast, our knowledge in respect to the more complex configuration of the ribonucleoprotein contained in these virions is surprisingly poor. Attempts will be made and methods will be developed to isolate and visualize the ribonucleoproteins of the retraviridae.

B. During the classification of two new virus isolates, both belonging to the family of retraviridae, a unique association of viral precursors with certain cellular organelles has been observed. Studies are initiated to investigate in detail the nature of the cellular involvement, especially in respect to intracellular movement of viral precursors.

Publications:

Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Cicmanec, J. L., Heine, U. and Kingsbury, E. W.: Comparison of lymphomas induced in owl monkeys with Herpesvirus saimiri (HVS), heated HVS and HVS genome carrying cells. In: Monograph 5th International Conference on Multiple Primary Malignant Tumors (ed. L. Severi), Division of Cancer Research, University of Perugia, Perugia, Italy, 721-729, 1975.

Ablashi, D. V., Oie, H. K., Armstrong, G. R., Didier-Fichet, M. L., Tronick, S. R., Heine, U. and Dalton, A. J.: Xenotropic Properties of an Isolate from Murine Rauscher Leukemia Virus in Primates. J. Med. Primatol. 5: 223-236, 1976.

Schultz, R., Ruiz, P., Chirigos, M. and Heine, U.: Establishment and Characterization of a Cell Line Derived from a Spontaneous Murine Lung Carcinoma (M109). In Vitro (In Press).

Dion, A. S., Heine, U. I., Pomenti, A. A., Korb, J. and Weber, G. H.: Electrophoretic analysis of the molecular weight of murine mammary tumor virus RNA. J. Virology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04814-06-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Endogenous Oncornavirus-like Particles of the Hamster

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. G. Suskind	Medical Director	LVC NCI
Other:	T. H. Marshall	Expert	LVC NCI
	S. O'Brien	Geneticist	LVC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Ultrastructural Studies Section

INSTITUTE AND LOCATION
Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to characterize endogenous oncornavirus-like particles of the hamster with respect to: 1) their infectivity and mode of proliferation; 2) their relationship to other oncorna viridae; 3) whether control mechanisms for the expression of this agent can be associated with cytogenetic abnormalities and specific chromosomal loci in somatic cell hybrids.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Characterization of intracisternal oncornavirus-like particles of the hamster ("R" particles).

1. Clarification of their possible association with cell cultures derived from human tumors.

2. Determination of their life cycle, infectivity and manner of release.

3. Determination of their relationship to other oncornaviridae.

4. Characterization of chromosomal loci possibly associated with control of expression of virion in somatic cell hybrids.

Methods Employed:

Electron microscopy, electron microscopic autoradiography, tissue culture techniques involving single cell cloning and cell fusion, somatic cell hybridization, cytogenetic techniques including chromosome banding and fluorescent staining techniques. Isozyme analysis on starch gel electrophoresis, ultracentrifugation, and density gradient centrifugation.

Major Findings:

The presence of intracisternal, virus-like particles of characteristic radial ultrastructure, initially observed in 3 cell cultures derived from surgically removed human meningiomas and gliomas could not be confirmed in 5 other cases of meningioma and in a total of 11 cell cultures derived mainly from gliomatous tumors obtained from the Office of Program Resources. Moreover, isozyme studies of one of the cultures containing "R" particles showed that the electrophoretic mobility both of lactic acid dehydrogenase and nucleoside phosphorylase coincided with that of hamster BHK cells. These results imply that at least 2 chromosomes of the cell line examined are of hamster origin. Also, further cytogenetic studies did not confirm the previously reported human karyotype, but pointed to characteristics of hamster cells.

Although these virus-like particles appear to be unrelated to human tumors, their occurrence as endogenous viruses of the hamster, of unknown life cycle and infectivity was considered of further investigative interest. By clonal selection a somewhat more abundant source of these particles was obtained so that they can be found electron microscopically in most cells and not as sporadic, incidental observations.

Morphologically, two types of proliferation into the cisternae of the endoplasmic reticulum were observed: in one, the nucleoid is formed by

budding from the cytoplasm into the cisternal lumen in a manner similar to that observed for murine intracisternal "A" particles. In the other, proliferation occurs by longitudinal fission, during which the outer membranes of virus particles fuse to enclose several incomplete nucleoids and then separate. Both types of proliferation are associated with incorporation of membrane-bound ribosomes into the nucleoid and under conditions of induction morphologically abnormal ribosomal aggregates have been observed in association with the budding process.

Recognizable "R" particles have not been observed outside the cisternae of the endoplasmic reticulum, either in the cytoplasm or at extracellular sites. Virus particles have not been recovered from ultracentrifugates of tissue culture fluid purified over a sucrose cushion. With the breakdown of the endoplasmic reticulum in degenerating cells, the virion appears to disintegrate into morphologically nonspecific granules that may be observed within cell debris.

After application of halogenated pyrimidines in conjunction with subsequent maintenance of these cultures in arginine deprived medium, increased proliferation by budding was induced. Under those conditions, some abnormal nucleoid cores without fully formed virion particles were encountered, but evidence for the extracellular release of these particles was not observed.

Attempts to characterize the budding virus particles by autoradiographic techniques under conditions of pulse labeling with tritiated nucleic acid precursors have not been successful so far, largely because of the low incidence of the virus particles.

Current efforts are directed towards determining whether a control mechanism of a possible virogene for this endogenous hamster virus can be associated with chromosomal loci in segregating somatic cell hybrids, and whether the presence of heterospecific chromosomes can suppress such oncornavirus-like particles. Results obtained so far are very preliminary.

Significance to Biomedical Research and the Program of the Institute:

Despite the negative implication of these findings with respect to the association of "R" particles with human tumors and the as yet limited insight gained into the life-cycle and function of these endogenous viruses of the hamster, it does appear possible to study the control mechanism for expression of this endogenous virus occurring in relative abundance in continuous culture.

Proposed Course:

Recent studies in somatic cell hybrids segregating mouse chromosomes point to a control mechanism for the expression of murine intracisternal type "A" particles located in acrocentric chromosomes. We would like to study whether a similar control mechanism can be demonstrated for "R" particles in certain

chromosomal loci of hamster cells using hamster-mouse somatic cell hybrids segregating hamster chromosomes. It is proposed to correlate the incidence of "R" particles with the segregation of chromosomes as determined by banding techniques and isozyme assay and with the distribution of chromosomal loci particularly for ribosomal gene activity (nucleolus organizer regions) and satellite DNA. We also wish to determine whether the presence of some heterospecific human chromosomes or chromosomal loci, e.g. for ribosomal genes or for interferon, may alter the expression of an endogenous virus of another species.

The possibility of developing other assay systems for this agent such as immune electron microscopy and reverse transcriptase assays will be explored.

Publications:

Connan, G., Suskind, R. G. and Rabotti, G. F.: Asynchronous Transcription of Cellular Ribonucleic Acids After Exposure to Low Doses of Actinomycin D in Chick Fibroblasts Transformed by Rous Sarcoma Virus. Biochimie (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04821-05-LVC

PERIOD COVERED July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetics and environmental factors in susceptibility to tumor viruses.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	G.J. Todaro	Medical Officer, Chief	LVC, NCI
OTHER:	R.E. Benveniste	Microbiologist	LVC, NCI
	R. Callahan	Staff Fellow	LVC, NCI
	C.J. Sherr	Medical Officer	LVC, NCI
	U.R. Rapp	Visiting Scientist	LVC, NCI
	R.N. Fabricant	Research Associate	LVC, NCI
	T.H. Marshall	Expert	LVC, NCI
	S.A. Sherwin	Research Associate	LVC, NCI
	T.I. Bonner	Expert	LVC, NCI

COOPERATING UNITS (if any)

Electronuleonics, Inc. Bethesda, Md.; Naval Biological Research Laboratory, Oakland, Cal.

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Office of the Chief

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A model for cellular malignant transformation was proposed by the demonstration of a type of molecule that acts as a product of an oncogene would be expected to act. These small proteins such as Epidermal Growth Factor, Nerve Growth Factor, and others act by binding to specific Growth Factor Receptors on the surface of target cells stimulating them to divide. Several new retroviruses were identified and characterized: (a) two type C viruses from Mus cervicolor, one related to the woolly monkey-gibbon ape type C virus, the other related to the type C virus from Mus musculus. (b) A new class of retrovirus from Mus cervicolor and Mus caroli which are unrelated to all previously known retroviral groups. (c) Primate type C viruses several Papio species (baboons) and Theropithecus. (d) A group D retrovirus from Presbytis (langur monkey) related but distinct from Mason-Pfizer Monkey virus. (e) Several isolates from Saimiri (squirrel monkey).

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To isolate and characterize endogenously and exogenously transmitted type C viruses from primates, man, and other animals.

2. To determine the mechanism by which cells control the expression and production of endogenous oncogenic viruses and/or viral information and antigens.

3. To study how agents such as chemical carcinogens, irradiation, and viruses act alone or in concert to facilitate cell transformation and derepress virus expression.

4. To study the biological characteristics of isolated RNA, DNA and other components from tumor viruses and their relationship to possible transformation of human cells.

5. To characterize the basis for the wide ranging susceptibilities of cells from various origins to infection by exogenously added oncogenic RNA viruses.

6. To study the relationship of "spontaneous transformation" of cells in culture and in vivo to the presence of endogenous or horizontally transmitted type C viruses or virally coded information.

7. To study the modes of transmission, perpetuation, and evolution of type C oncogenic viruses within and among animal species.

Methods Employed:

Standard techniques of tissue culture, biochemistry, molecular biology, molecular genetics, immunology and virology were used. These methods were adapted to the specific test systems.

Major Findings:

1. A model for cell transformation has been suggested that projects the type of products that may be expected from oncogenes and may serve to mediate the escape of normal cellular controls. One class of substances that may be considered are those growth hormones that stimulate cells to divide, are present in cells in minute amounts, and have normal physiologic functions in development and perhaps throughout the lifetime of an animal. Epidermal Growth Factor (EGF) is a sequenced protein weighing approximately 6,000 daltons. Its action has been documented in vivo. In cell cultures it is a potent stimulator of cell division. It operates by binding to specific membrane receptors. This receptor complex signals the nucleus to induce cellular DNA synthesis. Cells without EGF receptors are not stimulated by it.

Mouse and human EGF cross-react strongly. When normal murine cells are compared to various transformed cells, it is seen that only mouse sarcoma virus transformed cells are attended in their ability to bind exogenously added EGF. Mouse sarcoma virus transformed cells cannot bind added EGF while DNA virus transformed cells and chemically transformed cells continue to bind EGF very well. Non-transforming viruses do not affect the binding capacity of infected cells. It is proposed (according to experimental evidence) that sarcoma virus transformed cells produce a substance much like EGF which may be the sarcoma gene product itself. Cell growth in an organism may be controlled, in part, by a display of growth factor receptors on the surface of cells and the contribution of growth factors by cells that do not themselves respond to the factors. Thus, inappropriate production of growth factors or alterations in the quantity or quality of receptors in recipient cells could serve as a stimulus for cell division and inappropriate cell growth. Nerve Growth Factor (NGF) is another of these substances of interest. It has been found that sympathetic ganglia, embryonic sensory ganglia, mouse neuroblastomas, and human melanomas possess receptors for NGF.

2. Two isolates of type C viruses were obtained from Mus cervicolor. One of these isolates is immunologically related to the woolly monkey-gibbon ape group of infectious type C viruses. The other isolate shares antigenic determinants with type C virus of M. musculus. In addition, a new class of endogenous retrovirus was isolated from Mus cervicolor and Mus caroli. These viruses are morphologically distinct from all other retroviral classes and are unrelated by immunologic and nucleic acid homology to all murine type B and C viruses as well as members of other retroviral groups.

3. Different retroviruses have been isolated from various primate species by cocultivation of tissues with heterologous cells. These isolates include type C viruses from several species of baboons (Papio) and from the closely related genus, Theropithecus. In addition, a group D retrovirus has been isolated from a langur monkey (Presbytis) cell line. This isolate is related to Mason-Pfizer virus (MPMV), but can be readily distinguished from MPMV by its host range, antigenic properties, and by nucleic acid hybridization. Various isolates from squirrel monkey (Saimiri) cell lines have also been obtained. These latter viruses are endogenous to squirrel monkeys, and are present in multiple copies in the cellular DNA of all squirrel monkey cells and tissues.

4. The endogenous ecotropic virus of the low leukemic strains of mice C3H and BALB/c is poorly infectious and of low pathogenicity. Cells infected with these viruses produce gs antigen and express GCSA (Gross Cell Surface Antigen) on their membranes but not GIX and production of virus particles is exceedingly low. The level of gp70 produced in these cells is 10- to 100-fold below the level found in productively infected cells whereas p30 antigens are present in comparable quantities. Many of the cells that are infected with replication deficient virus segregate gs antigen negative cells in subsequent cell divisions. These poorly infectious viruses can be converted to a replication competent pathogenic form when grown in certain transformed

mouse cells. This conversion involves several mutations and possibly recombinational events. The replication competent virus is able to activate xenotropic virus in infected cells and is pathogenic in NFS mice.

5. The understanding of the functional characteristics of the type C viral proteins would be of major importance in studying the host cell-virus interactions. The major structural phosphoprotein of the avian and mammalian type C viruses as the specific homologous viral RNA binding protein was characterized.

6. A wide range of human tumor specimens were analyzed for the presence of infectious type C virus by techniques of in vitro cocultivation, thus far with negative results. Moreover, many of the same tissues were analyzed by nucleic acid hybridization for the presence of gene sequences related to those of the endogenous type C virus of baboons, the only endogenous virus isolated from primates. There is, at present, no firm evidence that this virus has been horizontally transmitted to man, thereby resulting in malignancy. Finally, a series of experiments were conducted to determine whether antibody-independent, complement-mediated viral lysis might prevent the spread or release of type C viruses in primates. However, such virolytic activity was detected in those primate species which do, in fact, release and horizontally transmit type C viruses.

Significance to Biomedical Research and the Program of the Institute:

These projects relate to the nature of the interaction between tumor viruses and the cells that contain them. Development of techniques in the model systems are frequently applicable to the search for human oncogenic viruses. The methods by which the Program can hope to detect or to prevent virus-induced tumors will depend on how the viruses produce their effect. Two very different models must be considered; they act via the addition of genetic information to the cell from the outside or via activation of previously repressed genetic information. Which of these is the more critical under natural conditions for cancer in man is of paramount importance in determining how best to interrupt or prevent this process.

The finding of NGF receptors of human melanomas provides a specific surface marker on this highly metastatic and aggressive type of neoplasm. This marker may provide a mechanism for specifically attacking this tumor by immunologic means in the clinical setting. The possibilities of using the finding for diagnostic purposes and tumor response during therapy remain to be thoroughly explored.

Proposed Course:

1. Vertebrate evolutionary relationships, in particular among the species of the primate suborder Anthropoidea (Old World monkeys, apes and man) can be further determined by molecular hybridization studies utilizing additional specific reagents which will be prepared to each of the major components of

the primate endogenous and infectious type C viruses. These reagents will also serve as tools to study the degree of expression of virus specific information in healthy humans and humans with tumors and its relationship to known primate type C viruses.

2. To develop additional approaches to recover transmissible endogenous type C viruses from primates, directed particularly toward human normal and malignant tissues, cultured cell strains and cell lines. Cocultivation techniques currently in use, combined with other available methods, will also be utilized further toward a resolution of the presence of type C viruses in man and the relationship of the viruses, if found, to known primate endogenous and infectious type C RNA viruses. These studies would yield information on their mode(s) of transmission, evolutionary relationships, and role in cancer causation in man. Intrinsic and extrinsic factors (such as environmental agents, chemical carcinogens, etc.) involved in susceptibility and resistance to oncogenic virus infection and their characterization could ultimately be applied to the development of in vitro tests to determine an individual's risk of overtly expressing virogene information and any fundamental differences which predispose some to cancer.

3. To characterize the mechanism of cellular regulation of endogenous virogene sequences for the development of virus derepression techniques to activate expression of type C viral information, induce virus production or malignant transformation in human cells.

4. To characterize the new virus isolate reported this year and determine whether these classes of viruses are associated with a form of neoplasia in animals or humans.

5. The genetic changes that are required for the virulence of endogenous mouse type C viruses will be further analyzed by serological and biochemical methods. Virulent viruses from rodents and primates will be used as activating viruses in superinfection activation experiments with rodent and primates in vitro and, where possible, in vivo. The ability of these viruses to act as cocarcinogens in in vitro transformation will be studied using a quantitative chemical carcinogenesis assay.

6. To study the correlation of NGF binding to human melanomas with the clinical course. The use of the NGF receptor as a possible means of inhibiting tumor growth will also be studied.

7. Techniques will be developed to obtain DNA probes specific to various regions of the genome of the endogenous baboon virus. These probes should allow DNA hybridization measurements to determine which viral genes are most conserved both between species and within the multiple copies of viral related sequence in the baboon. Probes specific to the most conserved regions of the viral genome may provide improved sensitivity for endogenous human viral sequences. The virus PP-REC, thought to be a recombinant virus containing

baboon, mink, mouse and rat viral information, will also be studied in order to determine which portions of the viral genome are specified by each species of viral information.

Publications:

Todaro, G.J.: Type C virogenes: Genetic transfer and interspecies transfer. In Crowell, R.L., Friedman, H. and Prier, J.E. (Eds.): Tumor Virus Infections and Immunity. Baltimore, Md., University Park Press, 1976, pp. 35-44.

Todaro, G.J., Benveniste, R.E., Sherr, C.J., Lieber, M.M. and Callahan, R.: Infectious primate type C virus group: Evidence for an origin from an endogenous virus of the rodent, Mus caroli. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research 1975. Basel, Switzerland, S. Karger, 1976, pp. 115-120.

Todaro, G.J., Sherr, C.J. and Benveniste, R.E.: Baboons and their close relatives are unusual among primates in their ability to release nondefective endogenous type C viruses. Virology 72: 278-282, 1976.

Todaro, G.J.: Type C virogenes: Modes of transmission and evolutionary aspects. In Neth, R., Gallo, R.C., Mannweiler, K., and Moloney, W.C. (Eds.): Modern Trends in Human Leukemia II. Munchen, Germany, J.F. Lehmanns Verlag, 1976, pp. 357-374.

DeLarco, J. and Todaro, G.J.: Membrane receptors for mouse leukemia viruses: Characterization using the purified viral envelope glycoprotein, gp71. Cell 8: 365-371, 1976.

Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Todaro, G.J. Type C viruses from Kirsten sarcoma-transformed mink cells cocultivated with primate cells and expressing p30 antigens related to feline leukemia virus. J. Virology 19: 346-358, 1976.

Sen, A., Sherr, C.J. and Todaro, G.J.: Specificity of in vitro binding of primate type C viral RNA and the homologous viral p12 core protein. Science 193: 326-328, 1976.

Todaro, G.J., Benveniste, R.E. and Sherr, C.J.: Interspecies transfer of RNA tumor virus genes: Implications for the search of "human" type C viruses. In Baltimore, D., Huang, A.S. and Fox, C.F. (Eds.): Animal Virology (ICN-UCLA Symposia on Molecular and Cellular Biology, Vol. 4). New York, New York, Academic Press, 1976, pp. 369-384.

Peries, J. and Todaro, G.J.: A hemagglutination assay for the primate syncytium-forming (foamy) viruses. J. Gen. Virol. 34: 195-199, 1977.

Callahan, R., Benveniste, R.E., Sherr, C.J., Schidlovsky, G. and Todaro, G.J.: A new class of genetically transmitted retrovirus isolated from Mus cervicolor. Proc. Natl. Acad. Sci. 73: 3579-3583, 1976.

Todaro, G.J., DeLarco, J. and Cohen, S.: Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor (EGF) to cells. Nature 264: 26-31, 1976.

Sen, A. and Todaro, G.J.: The genome-associated specific RNA binding proteins of avian and mammalian type C viruses. Cell 10: 91-99, 1977.

Fabricant, R.N., DeLarco, J.E. and Todaro, G.J.: Nerve growth factor receptors on human melanoma cells in culture. Proc. Natl. Acad. Sci. 74: 565-569, 1977.

Benveniste, R.E., Callahan, R., Sherr, C.J., Chapman, V. and Todaro, G.J.: Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: Conservation of virogene sequences in related rodent species. J. Virol. 21: 849-862, 1977.

Sen, A., Sherr, C.J. and Todaro, G.J.: Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. Cell 10: 489-496, 1977.

Todaro, G.J.: RNA tumor virus genes (virogenes) and the transforming genes (oncogenes): Genetic transmission, infectious spread and modes of transmission. Cold Spring Harbor Symposium on Quantitative Biology, (In Press).

Todaro, G.J.: RNA tumor virus genes (virogenes) and the transforming genes (oncogenes): Genetic transmission, infectious spread, and modes of transmission. J. Natl. Cancer Inst. (In Press).

Todaro, G.J.: Evidence for the interspecies transmission of tumor virus genes. In Gallo, R.C. (Ed.): Advances in Cancer Research: Cell Biology, Molecular Biology and Tumor Virology. Cleveland, Ohio, CRC Press, (In Press).

Callahan, R., Sherr, C.J. and Todaro, G.J.: A new class of murine retroviruses: Immunological and biochemical comparison of novel isolates from Mus cervicolor and Mus caroli. Virology (In Press).

Sliski, A.H., Essex, M., Meyer, C. and Todaro, G.J.: Feline oncornavirus associated cell membrane antigen (FOCMA): Expression in feline sarcoma virus transformed non-producer mink cells. Science (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04822-07-LVC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Utilization of Viral Markers for the Study of the Etiology and Control of Human Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. H. Levine	Medical Officer	LVC NCI
OTHER:	R. R. Connelly	Research Statistician	BB NCI
	R. Biggar	Medical Officer	LVC NCI
	C. W. Berard	Pathologist	LP NCI
	W. Wallen	Senior Staff	NINDS
	J. M. Easton	Medical Officer	LVC NCI
	D. V. Ablashi	Microbiologist	LDTV NCI
	R. B. Herberman	Medical Director	LCBGY NCI

COOPERATING UNITS (if any) Dr. F. Nkrumah, Univ. of Ghana; N. Mourali, Inst. Salah Azaiz, Tunis, Tunisia; V. Hyams, Armed Forces Inst. of Path.; S. H. Chan, Univ. of Singapore; J. Ho, Queen Eliz. Hosp., Hong Kong; P. Terasaki, UCLA, Los Angeles, Cal.; E. Perlin, Nat'l Naval Med. Ctr.

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION

Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to determine which viruses are specifically associated with certain human tumors and to use antigens relating to these viruses for studies on the etiology, prevention, early diagnosis and control of these malignancies. Current projects include: the separation of lymphomas into Epstein-Barr virus (EBV) associated and non-EBV associated tumors; the search for immune-response genes relating to EBV; the use of EBV antibody as a means of detecting susceptibility to cancer; the development of new assays to understand the mechanisms determining the outcome of EBV infection, and the identification of factors enhancing the dissemination of breast cancer.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To utilize assays measuring immunity to EBV in studies on the etiology and control of EBV-associated diseases, with particular emphasis on lymphoma and nasopharyngeal carcinoma.

B. To investigate the pathogenesis and treatment of inflammatory breast carcinoma in Tunisia as a model for aggressive breast cancer in the United States.

Methods Employed:

Project A

Cell mediated immunity (CMI) assays and additional serologic assays were developed and applied to studies of EBV immunity. For measurement of CMI, the leukocyte adherence inhibition (LAI) assay, the migration inhibition (MI) assay, and the lymphocyte stimulation (LS) assay were employed. Virion (V) and soluble (S) EBV antigens were utilized in each assay. In addition to the serologic assays measuring antibody to viral capsid antigen (VCA), early antigen (EA) and EBV nuclear antigen (EBNA), complement fixation to detect antibody to S antigen and antibody dependent lymphocyte cytotoxicity (ADLC) to detect antibody to membrane antigen (MA) were used. Patients with infectious mononucleosis (IM) were identified at local hospitals and student health centers. A registry of American nasopharyngeal carcinoma (ANPCR) was established and the registry of American Burkitt lymphoma patients (ABLR) was continued in order to obtain a population to compare clinically, epidemiologically, and virologically with the African Burkitt and Asian and African NPC patients. Blood samples were also collected from a variety of lymphoma patients, particularly those with American Burkitt's lymphoma and Hodgkin's disease, as well as normal and disease controls. Patients, family members and controls were typed for HL-A antigens to determine possible susceptibility to cancer.

Project B

Histologic sections from selected patients with inflammatory and non-inflammatory breast cancer were analyzed to determine if pathologic features could be identified and associated with clinical features. Skin testing was performed with dinitrochlorobenzene (DNCB) and with membrane extracts from breast cancer tissues and from cell lines derived from breast cancer tissues. These *in vivo* skin test measurements of cell-mediated immunity were correlated with *in vitro* cell-mediated and humoral immunity assays.

Major Findings:

Project A

A comparison of LAI, CMI and LS revealed that all assays were able to detect CMI to EBV, but the LMI was most reliable and sensitive. CMI to S antigen by all 3 assays developed later in the course of IM than CMI to V antigen. Lymphoma patients had augmented CMI response to EBV, contradicting the suggestion that elevated EBV titers in lymphoma were due to depressed CMI.

Serologic studies on a population-based group of Danish Hodgkin's disease (HD) patients and controls indicated the high EBV titers were specifically related to HD, antibody to other herpesviruses not being elevated. A possible relationship of titers to genetic factors was noted in family studies and in comparison of EBV titer and HL-A type. In one family where HD was diagnosed in two siblings, however, laboratory studies suggested that environmental factors were more important than genetic factors in the appearance of HD in this particular family.

Patients with NPC in countries with high, low, and intermediate incidence rates were found to have high EBV antibody levels by a battery of serologic assays. The VCA and EA assays were most useful in separating NPC patients from matched cancer controls, and IgA antibody titers to VCA had the greatest promise of being useful in diagnosis and/or prognosis. Differences between NPC patient groups by country of origin were observed, the high VCA and EA levels in Tunisian patients being of particular interest in view of the young age of onset of NPC in Tunisia. In vitro CMI assays were not able to distinguish NPC patients from controls in the small number of patients studied thus far, but skin testing with extracts of EBV-infected cell lines appeared to have potential in both diagnosis and prognosis.

Project B

Skin testing studies performed in collaboration with Dr. Nejib Mourali, Institute Salah Azaiz, indicated that the breast cancer patients with a fulminant form of breast cancer (poussè evolutive or PEV) were as likely to react to breast cancer related skin test antigens than patients with other forms of breast cancer, thus indicating that the fulminant form of disease (PEV 3) was not due to immunologic deficiency. This conclusion was supported by equivalent findings in EBV titers, DNCB skin test reactivity, and percentage of B and T cells.

Significance to Biomedical Research and the Program of the Institute:

Project A

The availability of nonhumoral assays measuring immunity to EBV provides a greater opportunity to define the factors modifying the effect of EBV in

different individuals. Additional diagnostic tools and predictors of morbidity may be developed from these new assays. The studies in American BL and NPC are important since a closer scrutiny of the same disease in geographically distinct areas allows a more careful look at the etiology by separating various factors. If American BL has the same etiology as African BL, it can be evaluated without concern for the role of malaria as a complicating factor. The findings in our studies that some cases of American BL have EBV within the tumor and have high antibodies to EBV while others do not have this EBV pattern parallel findings with African BL. The ability to separate Burkitt's tumor and potentially other lymphomas into diseases associated with different specific virus and tumor associated antigens may lead to identifying different viruses that produce the same morphological disease entity. The American Burkitt Lymphoma Registry is providing detailed information of great importance to oncologists. The geographical distribution and time-space clustering suggest an environmental contribution, perhaps a virus, to the etiology. Characterization of the clinical aspects of the disease is permitting chemotherapists to select drugs which prevent relapse more effectively. The finding of a specific genetic pattern associated with American Burkitt's tumor may explain why a ubiquitous virus would only produce this tumor under rare circumstances in the United States. Studies determining the multiple reasons for high EBV titers serve in conjunction with the various EBV assays to determine the usefulness of EBV serology in a variety of situations. The finding of high EBV titers in normal individuals in cancer families indicate that EBV serology may be used as a test for susceptibility to cancer. Correlation of certain EBV antibodies with clinical stage of the disease allows the use of these particular antibodies in monitoring the effect of conventional therapy as well as immunotherapy. The studies on the relationship of EBV antibodies to cell-mediated immunity are necessary to provide a better understanding of whether or not EBV is oncogenic and if so, how it is activated.

Project B

Comparative studies on breast cancer in Tunisia and the United States will lead to better understanding of factors related to the etiology and control of this tumor. Since the PEV form of breast cancer has been described in developing countries and disappears as the socio-economic conditions improve, the studies in Tunisia provide a unique opportunity to look at the environmental aspects associated with this tumor. These particular patients with fulminant disease also offer a better opportunity to isolate a human breast cancer virus and to correlate immunity to possible tumor virus associated antigens in a situation that contrasts markedly with that in Europe and the United States.

Proposed Courses:

Project A

Studies with the American Nasopharyngeal Carcinoma and Burkitt Lymphoma Registries will concentrate solely on those cases where adequate material can be obtained for laboratory studies. Because of the variability of pathologic material on many of these cases, it is necessary to separate those with well-fixed tissue sections and unequivocal pathologic features from those cases with atypical features and/or poor histological material for diagnosis. A comparative study of BL and NPC patients in endemic areas and the United States will be undertaken. Each patient will provide longitudinal serum samples and in vitro CMI assays will be performed when possible. The variety of reasons for elevated EBV titers will be pursued in order to determine which assays are of value for immunodiagnosis, monitoring of therapy, and studies of etiology. Skin testing with a number of EBV and tumor associated antigens in various neoplasms will be continued in order to determine if possible tumor specific and virus-related antigens can be used for monitoring conventional therapy and/or for immunotherapy.

Project B

In the next year, it is expected that 100 patients with breast cancer will be identified in Tunisia and approximately 20-30 will be studied in the United States. These patients will be followed longitudinally with skin testing, clinical examination, and serologic evaluation.

Publications:

Perlin, E., Levine, P. H., McCoy, J., Dean, J. and Herberman, R.: Hodgkin's Disease in Siblings: A Family Study. Oncology, Chapter 33: 116-118, 1976.

Hesse, J., Levine, P. H., Ebbesen, P., Connelly, R. R. and Mordhorst, C. H.: A Case-Control Study on Immunity to Two Epstein-Barr Virus-Associated Antigens and to Herpes Simplex Virus and Adenovirus in a Population-Based Group of Patients with Hodgkin's Disease in Denmark, 1971-1973. Int. J. Cancer 19: 49-58, 1977.

Ziegler, J. L., Magrath, I. T., Gerber, P. and Levine, P. H.: Epstein-Barr Virus and Human Malignancy. Ann. Int. Med. 86: 323-336, 1977.

Chan, S. H., Wallen, W. W., Soares, N. and Levine, P. H.: Lymphocyte responses to EBV-associated antigens in infectious mononucleosis and Hodgkin's and non-Hodgkin's lymphoma patients using the leukocyte adherence inhibition assay. Int. J. Cancer 19: 356-363, 1977.

Levine, P. H., Connelly, R. R., Heath, C., Caldwell, G., Terasaki, P. I., Mickey, W. R. and Fabrizio, P.: An Evaluation of Antibody Titers to the Epstein-Barr Virus as a Test for Susceptibility to Cancer. 3rd International Symposium on Prevention and Detection of Cancer (In Press).

Chan, S. H., Soares, N., Wallen, W. and Levine, P. H.: Detection of Immunity to possible viral and tumor associated antigens in breast cancer and melanoma using the leukocyte adherence inhibition assay. 3rd International Symposium of Prevention and Detection of Cancer (In Press).

Goodman, M. L., Levine, P. H. and Miller, D.: Nasopharyngeal Carcinoma Patients in Massachusetts. 3rd International Symposium on Prevention and Detection of Cancer. New York (In Press).

Levine, P. H.: Clinical Implications of Immunity to Oncogenic Viruses. In Rapp, F. and Hanna, M. G. (Eds.): Contemporary Topics in Immunobiology, Vol. 6 (In Press).

Levine, P. H., Stevens, D. A. and Lamellin, J. P.: Cellular Immunity Studies on NPC Patients and Controls. 2nd International Conference on Nasopharyngeal Carcinoma, Kyoto, Japan (In Press).

Pearson, G. R., Coates, H., Neel, B., Levine, P. H., Ablashi, D. and Easton, J.: Clinical evaluation of EBV serology in American NPC patients. 2nd International Conference on Nasopharyngeal Carcinoma, Kyoto, Japan (In Press).

Chan, S. G., Hong-Goh, E., Chew, T. S., Levine, P. H. and Simons, M. J.: Immunological Status of NPC Patients in Singapore. 2nd International Conference on Nasopharyngeal Carcinoma, Kyoto, Japan (In Press).

Ho, J. H. C., Chau, J. C. W. and Levine, P. H.: Longitudinal Studies on Delayed Hypersensitivity Reactions in Nasopharyngeal Carcinoma Patients in Hong Kong. 2nd International Conference on Nasopharyngeal Carcinoma, Kyoto, Japan (In Press).

Levine, P. H.: Is Burkitt's Lymphoma One Disease. Conference on Tumours of Early Life in Man and Animals, Perugia, Italy (In Press).

Ablashi, D. V., Easton, J. M., Levine, P. H., Krueger, G. R. K. and Connelly, R.: Immunological Comparison of Nasopharyngeal Carcinoma and Burkitt's Lymphoma from Relatively High- and Low-Risk Populations. Conference on Tumours of Early Life in Man and Animals, Perugia, Italy (In Press).

Wallen, W. C., Mattson, J. M. and Levine, P. H.: Detection of Epstein-Barr Virus Soluble Antigen by ELISA. J. of Infect. Diseases (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04823-04-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Expression of Endogenous Mammalian Type C Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C. J. Sherr	Medical Officer	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	R. E. Benveniste	Microbiologist	LVC, NCI
	S. J. O'Brien	Staff Fellow	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

New isolates of retroviridae have been obtained from primates and rodents. These include type C viruses isolated from baboons and a closely related genus, Theropithecus gelada; type C viruses isolated from Southeast Asian mice; a group D retrovirus isolated from a langur (Presbytis obscurus); and a distinctly new group of murine retroviridae isolated from M. cervicolor and M. caroli. With the exception of the langur virus, all of the other new isolates have been shown to be genetically transmitted in their species of origin.

A type C viral phosphoprotein (p12) has been characterized and shown to specifically bind to type C viral RNA from the homologous virus. The extent but not the specificity of binding is regulated by phosphorylation and appears to be under host cellular control.

Using techniques of somatic cell hybridization, a new gene on human chromosome six has been defined which regulates baboon type C viral replication in human X rodent cells. The new gene, designated Bevi, may be a preferred type C viral integration site in the human genome.

Project Description

Objectives:

To isolate and study the evolutionary relationships between genetically transmitted RNA tumor viruses (retroviruses); to define the functional role of type C viral proteins; to characterize host cell factors which influence the expression and replication of horizontally and genetically transmitted retroviruses.

Methods Employed:

Components of a wide variety of mammalian type C viruses are purified by standard biochemical techniques and employed for the preparation of specific antisera. Radioimmunoassays are used to test for the presence of viral antigens in extracts of animal tissues and in cultured cell lines. Evidence for type C viral expression is also obtained using biochemical assays for viral polymerases combined with antibody inhibition studies for "typing" enzymes. In collaboration with other investigators, these assays are supplemented by electron microscopic examination of tissues and cultured cells, molecular hybridization techniques, and a multiplicity of in vitro virologic assays (viral interference, rescue of sarcoma genomes, host range studies).

Major Findings:

Attempts to isolate infectious retroviruses have been primarily directed toward primate cells and tissues. Several independent type C isolates have been obtained from tissues of four species of baboons (Papio cynocephalus, P. hamadryas, P. anubis, and P. papio) and from a closely related genus, Theropithecus gelada. These isolates represent a group of closely related primate viruses whose genes have been genetically transmitted in primates for at least 30 million years. To date, endogenous type C viruses have not been isolated from other Old World monkeys and higher apes whose cells are known to contain type C viral genes and occasionally express viral antigens.

A new viral isolate has also been obtained from a Southeast Asian primate species, the langur (Presbytis obscurus). This virus, designated PO-1-Lu, is morphologically and biochemically related to the group D retrovirus isolated from rhesus monkeys (Mason-Pfizer monkey virus). PO-1-Lu and MPMV can be distinguished from one another, however, by immunological and nucleic acid hybridization criteria. The isolation of a virus related to MPMV from a second primate species whose natural habitat overlaps that of rhesus monkeys suggests that the group D retroviruses may be endemic to Southeast Asian primates.

Cocultivation of primate tissues with potentially permissive, indicator host cell lines from heterologous species has led to the isolation of recombinant type C viruses containing only a portion of the primate type C viral genome. The new isolates also contain RNA genomes partially homologous to the Kirsten sarcoma genome as well as to mink virogenic information. The

ability to obtain such recombinants from nonproductively transformed mink cells suggests that recombination offers one mechanism by which viruses can be obtained from cells which lack the ability to produce infectious, endogenous viruses.

New retroviruses which differ from other retroviridae have also been obtained from several Southeast Asian murine species (M. cervicolor and M. caroli). These viruses are endogenous to mice but can be readily distinguished by morphologic, biochemical, and immunological criteria from the type B and type C viruses previously isolated from mice. An analysis of the cellular DNA of rodents for sequences related to the genomes of these new viruses shows that this class of endogenous viral genes is highly conserved evolutionarily among the different rodent species.

The major phosphoprotein of murine type C viruses (p12) and its functional analogue in other type C viral groups (p16 or p19) has been shown to specifically bind to type C viral RNA purified from the homologous virus. The extent but not the specificity of binding has been shown to be regulated by the degree of phosphorylation of the p12 protein. The specificity of the protein-RNA interaction, the stoichiometry of the binding reaction, and the regulation of the extent of binding by post-translational phosphorylation of the protein all suggest that these proteins play a regulatory role in the viral life cycle.

Somatic cell hybridization techniques have been used in an attempt to localize the integration site(s) of the baboon type C virus in infected human X rodent cells. A major gene ("Bevi") on human chromosome six has been found which controls the expression of baboon type C virus in hybrid cells. Counter selection experiments with infected cells show that subclones segregating human chromosome six lose the ability to express the baboon type C virus. These results represent the first assignment of a human gene which regulates type C viral expression, and suggest that chromosome six is a preferred integration site for baboon type C virus in the human genome.

Significance to Biomedical Research and the Program of the Institute:

Our studies have confirmed the general validity of in vitro cocultivation methods for the isolation of new classes of mammalian retroviridae. By the use of rapid and sensitive assays for the presence of viral components, it has been possible to show that many species from which viruses have yet to be isolated contain endogenous viral genes and express viral antigens. Extensions of these techniques have helped to elucidate the functional role of type C viral proteins, and to define host cell genes which control viral expression.

Proposed Course:

Experiments are in progress to isolate new type C viruses from other primates with particular attention to human tissues. Additional immunological and biochemical assays for viral components are being developed to study the

functional role of viral components, and the interaction of infectious and endogenous viruses with host cells.

Publications:

Todaro, G.J., Sherr, C.J. and Benveniste, R.E.: Baboons and their close relatives are unusual among primates in their ability to release complete endogenous type C viruses. Virology 72: 278-282, 1976.

Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Todaro, G.J.: Type C viruses from Kirsten sarcoma-transformed mink cells co-cultivated with primate cells and expressing p30 antigens related to feline leukemia virus. J. Virol. 19: 346-358, 1976.

Todaro, G.J., Benveniste, R.E. and Sherr, C.J.: Interspecies transfer of RNA tumor virus genes: Implications for the search for "human" type C virus. In Baltimore, D., Huang, A.S. and Fox, C.F. (Eds.): Animal Virology (ICN-UCLA Symposia on Molecular and Cellular Biology). New York, Academic Press, 1976, Vol.4, pp. 369-384.

Callahan, R., Benveniste, R.E., Sherr, C.J., Schidlovsky, G. and Todaro, G.J.: A new class of genetically transmitted retravirus isolated from Mus cervicolor. Proc. Nat. Acad. Sci. USA 73: 3579-3583, 1976.

Sen, A., Sherr, C.J. and Todaro, G.J.: Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. Cell 10: 489-496, 1977.

Benveniste, R.E., Callahan, R., Sherr, C.J., Chapman, V. and Todaro, G.J.: Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: Conservation of virogene sequences in related rodent species. J. Virol. (In Press).

Sherr, C.J. and Benveniste, R.E.: Evolution and interspecies transfer of endogenous type C viral genes. 5th Reunion du Groupe D'Etudes des Oncornaviruses. Centre "Les Fontaines" - Chantilly, France (In Press).

Callahan, R., Sherr, C.J. and Todaro, G.J.: A new class of murine retroviruses: Immunological and biological comparison of novel isolates from Mus cervicolor and Mus caroli. Virology (In Press).

Lemons, R.S., O'Brien, S.J. and Sherr, C.J.: A new genetic locus, Bevi, on human chromosome six which controls the replication of baboon type C virus in human cells. Cell (In Press).

Todaro, G.J., Benveniste, R.E., Sherr, C.J., Schlom, J., Schidlovsky, G. and Stephenson, J.R.: Isolation and characterization of a new type D retrovirus from the Asian primate, Presbytis obscurus (spectacled langur). Nature (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04825-04-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physico-chemical Studies of Viral Nucleic Acids and Enzymes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. K. Haapala	Microbiologist	LVC NCI
OTHER:	L. A. Phillips	Microbiologist	LVC NCI
	R. H. L. Pang	Visiting Fellow	LVC NCI
	R. A. Ascione	Chemist	LDTV NCI
	W. G, Robey	Chemist	LDTV NCI

COOPERATING UNITS (if any)

J. C. Chermann	Biochemist	Pasteur Institute of Paris
F. Sinoussi	Biochemist	Pasteur Institute of Paris

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Nucleic acids of Moloney sarcoma virus (MSV) have been fractionated into subcomponents and used to detect virus-specific RNA in subcellular fractions of transformed cells. In addition, viral proteins have been detected in these fractions by SDS-PAGE after immunoprecipitates with antiserum prepared against MSV. In vitro synthesis of MSV specific proteins has been accomplished using RNA purified from subcellular fractions.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To characterize and utilize viral nucleic acids and protein as diagnostic and analytical tools for probing the nature and mechanism of viral oncogenesis.

B. To test various synthetic and biological compounds which inhibit viral replication in and/or transformation of cells *in vitro* in order to obtain products of therapeutic and/or heuristic value vis-a-vis the oncogenic process.

Methods Employed:

Standard biochemical, biophysical, cell and virus culture methods are used. These include ultracentrifugal, chromatographic, enzymological, nucleic acid hybridization, gel filtration, electrophoretic, immunological and *in vitro* protein synthesis techniques.

Major Findings:

A. 1. Last year I reported the successful fractionation of Moloney sarcoma virus into three components by sequential hybridization. This work has now been published by Frankel, Neubauer and Fischinger.

2. In collaboration with Drs. Pang and Phillips, the genetic constitution of the Gazdar isolate of MSV has been examined using the sequential hybridization technique described above. This work is submitted for publication.

3. Continuing studies of the phenotypic differences of the major group-specific protein of various MSV isolates have revealed a constancy of these phenotypes in various cell systems. We have recently published this work (Robey *et al.*, 1977).

4. In collaboration with Dr. Ascione, the putative site of MSV protein synthesis in cat cells has been determined (in preparation). In addition, RNA from these subcellular fractions of cat cells have been translated *in vitro* to give MSV specific protein.

B. Collaboration studies, with Drs. Chermann and Sinoussi, of inorganic and biological inhibitors of tumor viruses are continuing.

Significance of Biomedical Research and the Program of the Institute:

A. To determine the site of viral protein synthesis and the proteins is a step in elucidating the interaction of the cell and viruses in the oncogenic process. Understanding this process may well lead to new concepts in controlling this process.

B. Because of its obvious pragmatic value, the nature of virus specific inhibitors of both natural and synthetic origin has been pursued in the hope of obtaining inhibitors of therapeutic as well as heuristic value.

Proposed Course:

Studies on the interaction of virus and cells will be continued using the tools of nucleic acid hybridization and immunoprecipitation of viral protein. Putative intracellular proteins of virus origin will be studied with increased vigor because of its potential importance to the oncogenic process. Also, studies of potential viral inhibitors will continue in collaboration with Drs. Chermann and Sinoussi.

Publications:

Vande Woude, G. F., Robey, W. G., Oskarsson, M. K., Haapala, D. K., Fischinger, P. J., Naso, R. B. and Arlinghaus, R. B.: Properties of Moloney sarcoma virus specific p60 and its detection in transformed cells. In Clemmesen, J. (Ed.): Bibliotheca Haematologica No. 43. Basel, Switzerland, S. Karger AG, 1976, pp. 125-127.

Robey, W. G., Oskarsson, M. K., Vande Woude, G. F., Naso, R. B., Arlinghaus, R. B., Haapala, D. K. and Fischinger, P. J.: Cells transformed by certain strains of Moloney sarcoma virus contain murine p60. Cell 10: 79-89, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04826-05-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Mammalian Oncornaviruses: Mechanisms of Cell Transformation and Host Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. J. Fischinger	Hd, Virus Control Sect.	LVC NCI
OTHER:	A. E. Frankel	Senior Surgeon	LVC NCI
	D. K. Haapala	Microbiologist	LVC NCI
	S. Nomura	Microbiologist	LVC NCI
	J. Ihle	Hd, Immunobiology of Viral Carcinogenesis	NCI-FCRC
	C. F. T. Mattern	Microbiologist	LVD NIAID
	G. Robey	Staff Fellow	LTVG NCI
	G. F. Vande Woude	Hd, Virus Tumor Biochem. Sect.	LTVG NCI
Cooperating Units (if any)			
	D. Bolognesi	Assoc. Prof. Surgery	Duke University
	A. Chapman	Prof. of Anatomy	U. of Kansas
	F. de Noronha	Prof. of Veterinary Path.	Cornell U.
	W. Schäfer	Head, Max-Planck-Institut für Virusforschung	
	C. Croce	Professor	Wistar Institute
	F. Ruddle	Professor	Yale University
	R. Lerner	Professor, Scripps Clinic & Research Foundation	

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The properties, origins, and evolution of isolated subsets of MSV specific genes were examined. RNA transcription from MSV proviral DNA was studied in viral and chemically transformed cells, and revertants. MSV specified polyproteins were identified in mammalian transformed cells. Properties of various oncornaviral surface proteins such as gp71 and p15E were examined. Limited but specific immunity was achieved in three strains of mice against Friend MuLV after homologous, active gp71 immunization. Seroimmunotherapy was shown to be a better means of disease control.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To understand the variety of mechanisms and sarcoma virus specific nucleotide sequences which are involved in cell transformation induced by oncornaviruses.

B. To determine the mode of interaction and the nature of recombinational events among ecotropic and xenotropic viruses as related to the neoplastic process(es).

C. To isolate substructures and antigens of mammalian oncornaviruses, and to examine the effect of purified virus components on the life cycle of oncornaviruses in tissue culture and in selected mammalian hosts.

Methods Employed:

A. and B. Standard virological assays such as sarcoma virus focus formation, leukemia virus helper assay and focus induction in S+L- cells, viral interference, and virus antigen induction, are being used. Cloning of cells in microtiter dishes and as soft agar colonies with the usual biochemical modes of particle and protein isolation are employed routinely.

C. Biochemical methods such as Sephadex or hydroxyapatite column chromatography, velocity, and isopycnic density gradient centrifugation, specific enzyme assays, radioactive tracer studies, reverse transcriptase activity, gel immunodiffusion, preparative and analytical polyacrylamide gel electrophoresis, and the usual methods of extraction for proteins and nucleic acids are being employed. Molecular hybridization methods involving DNA·DNA and DNA·RNA hybrids are performed with C_0t and C_rt analyses.

Major Findings:

A. Complementary DNA, specific for the various regions of the MSV genome was isolated and separated into the MSV specific region; "sarc" and the region shared with Moloney MuLV; "common". "Sarc" nucleotide sequences were found as a single copy per haploid genome in normal mouse DNA but not in normal mouse RNA. Sequences related to "sarc" were also found in the DNA of related species at about one copy per haploid genome. The degree of relationship closely followed the normal taxonomic schema. "Common" sequences of MSV were found in normal mouse cell DNA only as related and multiple copies. Already in other mouse species these sequences disappeared and in the rat and related species the "common" sequences were absent. The rate of evolution of "sarc" which paralleled hemoglobin evolution rate was therefore totally different from the "common" sequences. A critical finding was that mouse cells transformed by a variety of agents not related to MSV did not express the RNA homologous to MSV "sarc" present in all mouse cells. Accordingly at the level of transforming virus there does not seem to be a unifying set of informational sequences whose function results in neoplasia.

A part of "common" MSV sequences is translated as an uncleaved polyprotein (p60). This translation occurred with great fidelity in the six heterologous species' cells transformed with the S+L- type genome of MSV. A second, cloned S+L- MSV variant expressed both p60 and a larger p70 precursor protein. The antigenic composition of determinants found on the p60 and p70 molecules has been analyzed.

The nature of cell reversion from the MSV transformed state continued to be studied. In the cloned cat cell system, MSV transformants lose the genome when they revert. Reversion was frequent prior to multiple cycles of cloning of S+L- cells; after about four cycles it appeared that essentially no more occurred. Revertants of mouse S+L- cells did express antigenic determinants and virus specific RNA. The degree of antigen relationships and nucleotide homologous suggested that the MSV genome is repressed in mouse cell revertants.

B. Studies on clone hybrid viruses such as HIX have shown that these have genetic, antigenic, and immunogenic properties compatible with the fact that these are genetically stable recombinant viruses. Recent collaborative studies indicated that recombination occurred within the major exterior glycoprotein (gp71) molecule itself based on two dimensional peptide analysis. Such viruses may be important as the real oncogenic variants. HIX virus was indeed found to be oncogenic in mice but not in cats.

Several other xenotropic type viruses were also cloned. Upon further antigenic analysis these were also found to be hybrids. Here, however, the recombination occurred in the group specific antigen region which is located on the other end of the molecule away from gp71.

C. The biological functions of gp71 isolated molecules were studied. gp71 itself was found to interfere, hemagglutinate and induce neutralizing antibody. The glycoprotein was digested with several endoglycosidase enzymes or various proteases. It was clear that the cell attachment, interference, and the neutralizing antigen site were all on the protein portion of the molecule. Carbohydrate was necessary only for hemagglutination.

A major collaborative effort with several laboratories was completed dealing with active immunization with Friend MuLV gp71 in three strains of mice, BALB/c, C57BL, and AKR. A summation would indicate that in the mouse immunization did result in a type specific response against the F-MuLV gp71 and a concomitant slight protection. However, in the C57/BL strain a derepression of endogenous Gross type virus occurred as determined by type specific anti Gross gp71 antibodies. In the AKR strain immunization was actually deleterious in that mice died of endogenous lymphoma sooner than expected.

Significance to Biomedical Research and the Program of the Institute:

A. It is now clear that nucleotide sequences involved in transformation of the cells of a single species can be molecularly unrelated. We have in hand now a well-characterized set of such sequences and can approach the question how this information affects the cellular program to render the cell neoplastic. In the present system at least two highly significant findings have implication for the field at large: (1) Normal cells have "sarc" type DNA sequences, but these don't have to be transcribed to RNA if the cells become transformed by agents other than MSV. (2) When nature made in related species other sarcoma viruses, some of which also have MuLV recombinant sequences, it did not use the analogues of MSV "sarc" sequences found in those species. Accordingly, the level of complexity has been found to be greater than anticipated.

B. Recent findings imply that recombinant MuLV type viruses may be the real causative agents in leukemogenesis. We have isolated such cloned viruses a year earlier than others and have found these to be oncogenic. It is clear from several of our isolates that stable genetic recombination among viruses is a common event in more than one region of the genome. The dissection of these interactions specifically in the region of recombination should clarify whether endogenous xenotropic viruses participate in the neoplastic process. The question of greatest significance that can now be answered is whether the recombinational event itself is integral to the neoplastic potential of these viruses or whether it somehow enhances the function of preexisting viral transforming genes.

C. The analysis of surface glycoprotein molecules of oncornavirus with regard to specific antigenic and biological functions has culminated in the obvious question of whether protection from leukemia could be achieved by active immunization. The findings of interspecies reactive determinants on gp71 molecules has lent credence to the possibility that other species may be protected even if the causative oncornavirus was not isolated. It was highly significant that active immunization was not the method of choice. Therefore, passive immunization with antibody directed against gp71 was attempted, and it was found in several laboratories around the world to be very promising in both the murine and the feline systems.

Proposed Course:

A. Further analysis of "sarc" and "common" sequences of MSV will be carried out. How do these sequences interact with the homologous and heterologous cellular DNA's? The intergration site(s) of "sarc" and "common" sequences is being considered. The role of multiple copies of MSV specific sequences during active virus replication is being investigated. Chromosomal segregation among interspecies cell hybrids and restriction enzyme mapping of cellular DNA are used to localize the MSV integration sites. Attempts will be made to identify and isolate the presumptive translational product of the "sarc" region of MSV. Nucleotide sequences of other sarcoma viruses will be analyzed. Temperature sensitive mutants will be isolated.

B. The role of recombinant viruses will be considered in neoplasia, specifically in species which do not appear to have such endogenous sequences. How common is recombination and how close or apart can the interacting viruses be is a major question. What are the specific recombinational regions in viral RNA and whether these differ from genes responsible for transformation of specific cells will be considered in detail.

C. Further analysis of precursor proteins of leukemia and sarcoma viruses with the specific emphasis on the recognition of potential transforming proteins will be attempted. Studies on protective mechanisms such as antibodies directed against virus specified cell surface proteins will be carried out. The relationships between passive protection by antibody directed against gp71 will be assessed in common with the probable necessity of identifying the autogenous immune response mounted by the protected animal. The question of such maternal antibody transfer will be examined.

Publications:

Nomura, S., Fischinger, P. J., Hartley, J. W. and Mattern, C. F. T.: Induction of a novel transforming virus (MSV-Z) from sarcoma positive leukemia negative 3T3FL mouse cells. Virology 69: 635-646, 1976.

Frankel, A. E., Haapala, D. K., Neubauer, R. L. and Fischinger, P. J.: Elimination of the sarcoma genome from murine sarcoma virus transformed cat cells. Science 191: 1264-1266, 1976.

Fischinger, P. J., Schäfer, W. and Bolognesi, D. P.: Neutralization of homologous and heterologous oncornaviruses by antisera against the p15(E) and gp71 polypeptides of Friend murine leukemia virus. Virology 71: 169-184, 1976.

Fischinger, P. J., Ihle, J. N., Bolognesi, D. P. and Schäfer, W.: Inactivation of murine xenotropic oncornavirus by normal mouse sera is not immunoglobulin-mediated. Virology 71: 346-351, 1976.

Nomura, S. and Fischinger, P. J.: Rapid screening assay for revertants of MSV-transformed cells. In Prescott, D. M. (Ed.): Methods in Cell Biology. New York, Academic Press, Inc., 1976, Vol: 14, pp. 229-236.

Schäfer, W., Claviez, M., Frank, H., Hunsman, G., Moennig, V., Schwarz, H., Thiel, H. J., Bolognesi, D. P., Green, R. W., Langlois, A. J., Fischinger, P. J. and De Noronha, F.: Mammalian C-type oncorna viruses. Relationships between structural virus and cell surface and their possible significance in immunological defense mechanisms. In Clemmesen, J. (Ed.): Bibliotheca Haematologica, No. 43. Basel, Switzerland, S. Karger AG, 1976, pp. 88-96.

Vande Woude, G. F., Robey, W. G., Oskarsson, M. K., Haapala, D. K., Fischinger, P. J., Naso, R. B. and Arlinghaus, R. B.: Properties of Moloney sarcoma virus specific p60 and its detection in transformed cells. In Clemmesen, J. (Ed.): Bibliotheca Haematologica No. 43. Basel, Switzerland, S. Karger AG, 1976, pp. 125-127.

Frankel, A. E., Neubauer, R. L. and Fischinger, P. J.: Fractionation of DNA nucleotide transcripts from Moloney sarcoma virus and isolation of sarcoma virus specific complementary DNA. J. Virol. 18: 481-490, 1976.

Frankel, A. E. and Fischinger, P. J.: Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc. Natl. Acad. Sci. USA 73: 3705-3709, 1976.

Ihle, J. N., Collins, J. C., Lee, J. C., Fischinger, P. J., Moennig, V., Schäfer, W., Hanna, M. G., Jr. and Bolognesi, D. P.: Characterization of immune response to the major glycoprotein (gp71) of Friend leukemia virus. I. Response in BALB/c mice. Virology 75: 74-87, 1976.

Ihle, J. N., Lee, J. C., Collins, J. C., Fischinger, P. J., Pazmino, N. H., Moennig, V., Schäfer, W., Hanna, M. G., Jr. and Bolognesi, D. P.: Characterization of the immune response to the major glycoprotein (gp71) of Friend leukemia virus. II. Response in C57BL/6 mice. Virology 75: 88-101, 1976.

Ihle, J. N., Collins, J. C., Lee, J. C., Fischinger, P. J., Pazmino, N., Moennig, V., Schäfer, W., Hanna, M. G., Jr. and Bolognesi, D. P.: Characterization of the immune response to the major glycoprotein (gp71) of Friend leukemia virus. III. Influence in endogenous MuLV-mediated pathogenesis. Virology 75: 102-112, 1976.

Frankel, A. E., and Fischinger, P. J.: Rate of divergence of cellular sequences homologous to segments of Moloney sarcoma virus. J. Virol. 21: 153-160, 1977.

Schäfer, W., Fischinger, P. J., Collins, J. J. and Bolognesi, D. P.: Role of carbohydrate in biological functions of Friend murine leukemia virus gp71. J. Virol. 21: 35-40, 1977.

Robey, W. G., Oskarsson, M. K., Vande Woude, G. F., Naso, R. B., Arlinghaus, R. B., Haapala, D. K. and Fischinger, P. J.: Cells transformed by certain strains of Moloney sarcoma virus contain murine p60. Cell 10: 79-89, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04828-08-LVC

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Immunology of Animal and Human Tumors, with Emphasis on Virus-Induced Mammary Neoplasia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	E. J. Plata	Microbiologist	LVC, NCI
OTHER:	T. Aoki	Medical Officer	LVC, NCI
	G. J. Todaro	Medical Officer, Chief	LVC, NCI
	J. Schlom	Microbiologist	LVC, NCI
	P. H. Levine	Medical Officer	LVC, NCI

COOPERATING UNITS (if any)

V.W. Hollis, Howard University, Washington, C.D.; D. Moore, Institute for Medical Research, Camden, N.J.; L.M. Boot, Netherlands Cancer Institute, Amsterdam; A. Duenas, Univ. del Valle, Cali, Colombia, S.A.

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Natural antibodies associated with mammary tumor virus infection and neoplasia were characterized and their appearance was examined in relationship with the onset of malignancy. Collaborative studies concerned characterization of viral and cellular antigens associated with tumors and their role in the tumor host's response in breast cancer, lymphoma and leukemia.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To develop new or to adapt current methods for the detection and characterization of viral or cellular antigens associated with neoplasia.

2. To study the humoral and cellular immune responses of the host to autochthonous and syngeneic tumors.

3. To explore applicable models for immunologic diagnosis, immunotherapy, or possible prophylaxis of tumors.

Methods Employed:

Standard immunological, animal, biological, biophysical and cell culture techniques. In most cases, these methods were modified sufficiently to render them more sensitive or more efficient for the particular studies.

Major Findings:

At least five MTV-associated antigen specificities detected in natural antibodies of MTV-positive mouse strains were resolved and partially characterized. Two of the comparable antigens also were isolated by solubilization and are continuing to be defined. The antibodies appeared spontaneously with age and were greatly enhanced when either spontaneous or transplanted mammary tumors developed in mice. This enhancement was not detectable when the mice were bearing tumors other than mammary cancer.

Distinct antibodies were found against normal thymocytes, murine leukemia virus, mammary tumor virus, L-cell, and fetal antigens. By comparison, tumor specific antibodies were found in relatively minor quantities. The significance of the natural antibodies to the pathogenesis of tumors and the antibodies' relationship to host expression of oncogenic viruses continue under study.

Significance to Biomedical Research and the Program of the Institute:

The characterization of natural antibodies associated with virus-induced tumors is significant because it clarifies any relationship between virus expression, malignant transformation, and the mechanism of the host immune response to tumors. These studies and the concomitant ones on the associated antigens will serve to instruct in the development of methods for early diagnosis for viral expression and tumor onset, and provide models for the advance study of the interaction of the host and the tumor.

Proposed Course:

To continue the characterization of tumor specific and tumor associated antigens in order to produce test quantities of each. To test the protective and/or enhancing capacity of monospecific active and passive immunization.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04829-03-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Molecular Structure and Expression of Endogenous Retroviral Genomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. Callahan	Staff Fellow	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	R. E. Benveniste	Microbiologist	LVC, NCI
	C. J. Sherr	Medical Officer	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Two isolates of type C viruses were obtained from Mus cervicolor. One of these isolates is immunologically related to the woolly monkey-gibbon ape group of infectious type C viruses. The other isolate shares antigenic determinants with type C virus of M. musculus. In addition, a new class of endogenous retrovirus was isolated from Mus cervicolor and Mus caroli. These viruses are morphologically distinct from all other retroviral classes and are unrelated by immunologic and nucleic acid homology to all murine type B and C viruses as well as members of other retroviral groups.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To determine the origin and relationship by nucleic acid homology of endogenous retroviruses isolated from different mammalian species and to study the extent and factors regulating the expression of the endogenous type C viral genome.

Methods Employed:

Molecular hybridization is employed to fractionate and compare viral genomes. Biochemical detection of virus and the expression of viral genomes in vivo include the reverse transcriptase assay, immunological assays for the gs antigen and molecular hybridization for viral RNA synthesis.

Major Findings:

During the past year several new isolates of murine retroviruses have been obtained from the Southeast Asian murine species Mus caroli and Mus cervicolor. These viruses can be classified into two general groups on the basis of immunological, biochemical, and morphological criteria.

The first group is composed of two subgroups of type C viruses isolated from Mus cervicolor. The first subgroup is immunologically related to the woolly monkey type C virus and a type C virus isolated from Mus caroli. This virus has been designated the M. cervicolor C I virus. The second subgroup is immunologically related to but can be distinguished from standard Mus musculus type viruses. This virus has been designated M. cervicolor C II virus. These two subclasses of type C virus can be further distinguished by their host ranges. The C I virus replicates well on tissue culture cell lines derived from either rabbit or cat. The C II virus replicates only on tissue culture cell lines derived from either M. musculus or M. caroli. Neither class of virus can replicate on M. cervicolor tissue culture cell lines. Sequences related to the viral genomes are present in multiple copies in the cellular genome of M. cervicolor. Less related viral sequences are found in other rodent species including other species of Mus and rats.

The second group of retroviruses exhibit properties which distinguish them from all other types of retroviruses previously described. Isolates have been obtained from M. cervicolor (M432) and M. caroli (M832). Each replicates in tissue culture cell lines derived from M. musculus but not in cell lines derived from the species of origin and were shown by immunologic and nucleic acid hybridization to be highly related but not identical. These viruses contain a 70S RNA genome which can be dissociated into 30S subunits. There are at least six virion associated proteins which are presumed to be encoded by the viral genome. These include the reverse transcriptase, two envelope proteins (gp65 and gp32), and three internal structural proteins (p24, p16, and p12). The major internal protein is p24. The p12 protein is phosphorylated.

The properties which distinguish this group of viruses from other retrovirus types include a magnesium-dependent reverse transcriptase with a molecular weight of 70,000 daltons; this differentiates the new isolate from the type C viruses which contain manganese-dependent polymerases and also from MMTV which contains a 100,000 dalton polymerase. The molecular weights of the structural proteins of the M432 isolate also differ from those of murine type B and type C viruses. Moreover, immunologic studies performed with the major internal structural protein (p24) or with the reverse transcriptase have not demonstrated cross-reactions with the homologous proteins of murine endogenous type B and C viruses as well as other retroviral groups. Nucleic acid hybridization data (Table 4) show no detectable sequence homology between the RNA genome of M432 and the genomes of other murine type B and C viruses, while all of the murine type C viruses so far tested (ecotropic, xenotropic, amphotropic) show sequence homology with one another.

Significance to Biomedical Research and the Program of the Institute:

The properties of the M. cervicolor C I virus supports previous work from this laboratory that suggests the origin of the woolly monkey-gibbon ape group of infectious type C viruses is the genus Mus. Further, the ability to distinguish between the C I and C II viruses immunologically as well as the lack of nucleic acid homology between them suggest that they may represent two subclasses of endogenous murine type C viruses.

The new class of murine retroviruses isolated from M. cervicolor (M432) and M. caroli (M832) are endogenous to the genus Mus and other more distantly related rodents. Whether this class of viruses is associated with a form of neoplasia in these species is unknown at this time. However, the existence of this class as well as the two distinguishable classes of endogenous type C viruses (C I and C II) and the endogenous murine type B viruses suggests that a significant portion of the murine cellular genome (at least 0.01%) is composed of retroviral genomes.

Proposed Course:

First, determine whether the C I, C II and the new class of retroviruses are pathogenic for susceptible laboratory animals. Second, test M. cervicolor mice of different ages for expression of viral related proteins and RNA. Third, further characterize the replicative cycle of the M432 virus.

Publications:

Callahan, R., Lieber, M.M., Todaro, G.J., Graves, D.C. and Ferrer, J.F.: Bovine leukemia virus genes in the DNA of leukemic cattle. Science 192: 1005-1007, 1976.

Callahan, R., Benveniste, R.E., Sherr, C.J., Schidlovsky, G. and Todaro, G.J.: A new class of genetically transmitted retravirus isolated from Mus cervicolor. Proc. Natl. Acad. Sci. USA 73: 3579-3583, 1976.

Todaro, G.J., Benveniste, R.E., Sherr, C.J., Lieber, M.M. and Callahan, R.: Infectious primate type C virus group: Evidence for an origin from an endogenous virus of the rodent, Mus caroli. In Clemmesen, J. and Yohn, D.S. (Eds.): Comparative Leukemia Research 1975, Bibl. Haemat., No. 43. Karger, Basel, 1976, pp. 115-120.

Benveniste, R.E., Callahan, R., Sherr, C.J., Chapman, V. and Todaro, G.J.: Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: Conservation of virogene sequences in related rodent species. J. Virol. (In Press).

Callahan, R., Sherr, C.J. and Todaro, G.J.: A new class of murine retroviruses: Immunological and biochemical comparison of novel isolates from Mus cervicolor and Mus caroli. Virology (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04832-03-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Tumor Virus-associated Surface Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	P. B. Brandchaft	Staff Fellow	LVC NCI
Other:	T. Aoki	Head, Immunology Section	LVC NCI

COOPERATING UNITS (if any)

A. B. White	Cell Biologist	WRAIR, WRAMC

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Immunology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the immune response to tumor-associated surface antigens. One area being pursued is the biochemical and immunological characterization of antigens extracted from tumor cells, using 3M KCl. Another is the analysis of the cellular and humoral immune responses in mice, following immunization with tumor antigens.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To isolate and characterize tumor-associated surface antigens extracted from Moloney murine leukemia (M-MuLV)-induced mouse leukemia cells.

2. To analyze the immune response to antigens on virus-induced tumor cells.

Methods Employed:

1. Antigens were solubilized from cells using 3M KCl. Antigen activity was measured by inhibition of the C'-dependent ^{51}Cr-releasing assay, immuno-agar gel diffusion, immunoprecipitation, and other serological assays.

2. Cell-mediated immunity was determined by lymphocyte-mediated cytotoxicity assays (^{51}Cr-release and ^{125}IUdR-release). Humoral immunity was measured by C' dependent ^{51}Cr-release cytotoxicity, virus neutralization (neutralization of MuLV helper activity in the S+L- focus-forming assay), immunoelectron microscopy. Resistance to tumor challenge after immunization was also studied.

Major Findings:

1. Soluble antigen preparations from M-MuLV-induced leukemia cells were fractionated by Sephadex G-200 column chromatography. Pooled fractions, corresponding to a molecular weight range of 25,000 to 45,000 daltons, contained most of the antibody-combining activity (as determined by inhibition of C' dependent serum cytotoxicity using an antiserum prepared against allogeneic tumor cells). This fraction also contained the virus structural protein p30. Whether the antigen which inhibits the cytotoxic activity of the anti-tumor cell antiserum is actually p30 is being investigated. Antigens which eluted from the Sephadex G-200 column in fractions corresponding to a molecular weight of 50,000 to 90,000 daltons were able to inhibit cytotoxic activity of rabbit anti-gp70 (M-MuLV) against MBL-2 cells.

2. Preliminary results suggest that immunization with antigens in the 25,000 to 45,000 dalton molecular weight fraction, as well as with those which are eluted with the void volume of the column, can protect syngeneic mice against tumor cell challenge.

The cellular and humoral immune responses to M-MuLV associated tumor antigens were analyzed after immunization of C57BL/6 mice with an allogeneic tumor induced by Moloney murine sarcoma virus. The kinetics of the cell-mediated cytotoxic response was measured by both the ^{125}IUdR- and ^{51}Cr-releasing assays. Activity of lymphocytes in the thymus or peripheral lymph nodes remained low. Activity was greater with lymphocytes from spleen

or mesenteric lymph nodes. Both assay methods detected a biphasic pattern in splenic lymphocytes with peaks at 2 weeks after immunization, and again 1 to 3 weeks later. The results of both assays suggested that cytotoxic lymphocytes in both peaks were primarily T cells; however, the data from the ^{51}Cr-releasing assay indicated that B cells were also functioning as cytotoxic effector cells. The kinetics of the humoral antibody response was analyzed using three different assays: C' dependent serum cytolysis, virus neutralization, and immunoelectron microscopy. Differences in the time of appearance of humoral antibody as well as in the persistence of antibody levels suggest that several types of humoral antibodies were produced in response to immunization with the tumor cells.

The immune response to M-MuLV-induced tumor cells (MBL-2) was analyzed in terms of the induction of cell-mediated immunity, humoral antibody formation, and resistance to tumor cell challenge. X-irradiated MBL-2 cells, which in some experiments were also treated with Vibrio cholera neuraminidase (VCN), were inoculated intraperitoneally into C57BL/6 mice. Immunization with either VCN-treated or untreated MBL-2 cells conferred tumor immunity, although mice receiving untreated cells were more resistant. The greatest protection was seen in both groups when the tumor cell challenge was given six weeks after immunization. The cell-mediated cytotoxicity response first occurred at 18-20 days after immunization; after this time, several peaks of cytotoxic activity were seen. Cytotoxic humoral antibody was first detected 20-30 days after immunization and persisted for at least 3 months. These results suggest that the highly lethal character of this tumor may be due to some extent to the late appearance of immunity.

Significance to Biomedical Research and the Programs of the Institute:

Cancer patients cannot usually mount an effective immune response to their own tumors. By investigating the immunological activities of solubilized tumor antigen and the immune response to tumor cells in experimental systems, we may better understand how to manipulate the immune response to effectively reject the tumor. Our finding that immunization against tumor cell challenge is most effective when the challenge dose is given 6 weeks after immunization, suggests that successful immunotherapy may depend upon carefully controlled immunization schedules.

Preliminary data suggest that certain soluble components of tumor cells are capable of inducing tumor immunity. Characterization of these components in experimental systems should aid in the detection of similar components in human tumors.

Proposed Course:

1. To analyze biochemically and immunologically, soluble antigens from leukemia cells.

2. To study the immune response in vitro and in vivo to tumor antigens.

3. To evaluate the role of viral proteins in the immune response to virus-induced tumors.

Publications:

Aoki, T., Liu, M., Walling, M. J., Bushar, G. S., Brandchaft, P. B. and Kawakami, T. G.: Specificity of naturally occurring antibody in normal gibbon serum. Science 191: 1180-1183, 1976.

Brandchaft, P. B., Aoki, T. and Silverman, T. N.: Immunogenic and immunosuppressive components in soluble preparations from Moloney murine leukemia virus-induced tumor cells. Int. J. Cancer 17: 678-685, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04836-03-LVC

PERIOD COVERED
July 1, 1976 to September 25, 1976

TITLE OF PROJECT (80 characters or less)

Effects of Transformation on the Interactions of Growth Factors and Their Membrane Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. E. De Larco	Senior Staff Fellow	LVC, NCI
OTHER:	R. Callahan	Staff Fellow	LVC, NCI
	U. R. Rapp	Visiting Scientist	LVC, NCI
	R. N. Fabricant	Research Associate	LVC, NCI
	T. H. Marshall	Expert	LVC, NCI
	G. J. Todaro	Medical Officer and Chief	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The relationships between sarcoma virus transformation and the expression of available membrane receptors for several polypeptide ligands were examined. Transformation of cells with either murine or feline sarcoma viruses specifically abolishes the binding of epidermal growth factor (EGF), a polypeptide growth factor, to its cellular membrane receptors. While the binding of three other polypeptide ligands, fibroblast growth factor (FGF), multiplication stimulating activity (MSA), and viral glycoprotein, to their membrane receptors were not decreased in murine or feline sarcoma transformed cells.

Epithelioid and fibroblastic clones were isolated from the same source, a normal rat kidney line, and were characterized with respect to their polypeptide ligand receptors and the effect of sarcoma transformation on their expression. The epithelioid clones bound eight times more EGF per cell than the fibroblastic clones. Both cell types upon transformation showed a sharp decrease or a total loss of measurable EGF receptors. The fibroblastic clones, however, bound more MSA than the epithelioid clones, and the MSA binding was independent of murine or feline sarcoma transformation.

Project No. Z01-CP-04836-03-LVC

Project Description

Objectives:

The advancement of basic knowledge concerning the relationships between polypeptide growth factors and cancer with the hope of eventually being able to understand and control neoplastic diseases *in vivo*.

Methods Employed:

Several polypeptide growth regulators were purified. These purified polypeptides were radioiodinated and binding assays developed to quantitate and characterize the membrane receptors for these ligands. Methods were developed for characterizing viral glycoprotein binding to freshly prepared tissues.

Major Findings:

Murine or feline sarcoma virus transformation specifically perturbs the EGF receptor system and not the receptors for the other ligands examined. A binding assay was developed for MSA so that cellular monolayers could be used rather than membrane preparations. Epithelial cells were shown to have more membrane receptors for EGF than the fibroblasts from the same source. The fibroblasts, however, bound more MSA than the epithelioid cells. Both the epithelioid and fibroblastic clones can be transformed by sarcoma virus; however, the fibroblastic clones were transformed much more efficiently than the epithelioid tested from the same tissue.

Publications:

De Larco, J. and Todaro, G.J.: Membrane receptors for murine leukemia viruses: Characterization using the purified viral envelope glycoprotein, gp71. *Cell* 8: 365-371, 1976.

Todaro, G.J., De Larco, J.E. and Cohen, S.: Transformation by murine and feline sarcoma viruses specifically blocks binding of epidermal growth factor (EGF) cells. *Nature*, 264: 26-31, 1976.

Fabricant, R.N., De Larco, J.E. and Todaro, G.J.: Nerve growth factor receptors on human melanoma cells in culture. *Proc. Natl. Acad. Sci. USA* 74: 565-569, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04840-07-LVC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Pathogenesis of Virus-Associated Tumors in Humans and Subhuman Primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Leiseca	Res. Biologist	LVC NCI
OTHER:	P. H. Levine	Medical Officer	LVC NCI
	E. J. Plata	Microbiologist	LVC NCI

COOPERATING UNITS (if any)

Dr. David Martin, Litton Bionetics, Inc., Kensington, Md.
Dr. T. Crandall Alford, George Washington University

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION

Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The project seeks to study the pathogenesis of virus-associated tumors in man, particularly breast cancer and lymphoma, with an attempt to utilize nonhuman primate models whenever possible. The studies are designed to provide information as to the possible viral etiology and means of controlling the tumors under evaluation.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Project A

To evaluate patients with "inflammatory breast cancer" to determine whether this is a definable entity and whether studies of this aggressive form of breast cancer can provide information as to the etiology and control of breast cancer in general.

Project B

To evaluate non-human primates as models for studies on the etiology and control of human cancer.

Methods Employed:

Project A

Patients with the clinical diagnosis of inflammatory breast cancer were sought from university hospitals in New York and Washington. Clinical information was abstracted from the charts and, when patients were available, blood samples were obtained for immunological evaluation. When possible, the patient was skin tested with standard antigens in order to evaluate their immune response.

Project B

Follow up information was obtained regarding rhesus monkeys and chimpanzees that had previously been inoculated with Epstein-Barr virus. If the animals were alive, their clinical status was documented and if the animals died, the cause of death was ascertained and pathology reports collected. Serum samples were requested from animals still under observation by investigators throughout the United States and Puerto Rico.

Major Findings:

Project A

Patients with the inflammatory form of breast cancer were found to have a shorter survival than those with supposed noninflammatory breast cancer. Slides were collected for evaluation by a group of pathologists to compare with patients with so-called inflammatory breast cancer (Pousseė evolutive) in Tunisia to determine if inflammatory breast cancer is a pathological entity.

Project B

A followup of the four chimpanzees and 49 rhesus monkeys inoculated with infective EBV revealed no evidence of lymphoma up to seven years after inoculation. Four of the animals inoculated were noted to have persistently high antibody titers to the Epstein-Barr virus. Evaluation of the information on this project suggested that rhesus monkeys could be used in limited studies on the oncogenicity of Epstein-Barr virus but that more careful selection of animals, including studies of genetic markers and serum antibody status, was necessary prior to inoculation with EBV.

Significance to Biomedical Research and the Program of the Institute:

Project A

Breast cancer is a leading cause of death in women and an extensive effort is underway in the National Cancer Institute to determine the etiology and control of this entity. The inflammatory form of breast cancer, which has an exceedingly poor prognosis and at the present time is mainly treatable by chemotherapy, serves as a model for the chemotherapy of breast cancer in general because of its resistance to most conventional measures. The report of inflammatory breast cancer in Tunisia suggests that certain environmental and genetic features are responsible for the high incidence of inflammatory breast cancer in that area. The identification and evaluation of patients in the United States with inflammatory breast cancer, estimated to be no more than 5% of the total cases of breast cancer in the U.S., may provide information as to the etiology of this particular entity and, in addition, will provide a specific group that can be studied in regard to immune response and control of this disease.

Project B

Circumstantial evidence implicates Epstein-Barr virus as the cause of Burkitt's lymphoma and nasopharyngeal carcinoma in humans. The availability of a nonhuman primate model could accelerate studies on the pathogenesis of EBV infection and provide clues as to why the virus causes apparent infectious disease in some humans, no clinical disease in other humans, and possibly cancer in other humans. Rhesus monkeys are easily bred in captivity and are sufficiently closely related to humans to allow investigation as to the pathogenesis of EBV infection. The response of rhesus monkeys to immuno-suppression and immuno-stimulation is very close to humans and these animals are also susceptible to malaria infection, a presumed cofactor in the etiology of Burkitt's lymphoma in Africa.

Proposed Course:

Project A

Approximately 100 patients with the clinical diagnosis of inflammatory breast cancer will be identified and will be matched with approximately 100 controls who do not have inflammatory signs on disease presentation. Slides from these patients will be evaluated under code by pathologists in an attempt to determine if inflammatory breast cancer is a pathologic entity. In addition, an attempt will be made to initiate immunologic studies to determine whether the clinical appearance of inflammatory breast cancer has an immunologic basis.

Project B

Primates inoculated with EBV will continue to be monitored for the appearance of lymphoma. Attempts will be made to obtain blood samples to determine if the antibodies to EBV are maintained and if there is cellular immunity to EBV. Utilizing a technique developed by the investigator, rhesus monkeys will be depleted of T-cells by thoracic duct cannulation, inoculated with infective EBV, and their immune response will be compared to inoculated animals not T-cell depleted.

Publications:

Martin, D. A. and Leiseca, S. A.: A Surgical Approach for Cannulation of the Thoracic Duct of the Rhesus Monkey (Macaca mulatta): Thoracic Approach. Laboratory Animal Science (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04841-03-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
The Structure and Distribution of the Nucleic Acid Sequences in the Moloney Sarcoma Virus

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. E. Frankel	Senior Surgeon	LVC NCI
OTHER:	P. J. Fischinger,	Hd. Virus Control Section	LVC NCI

Cooperating Units (if any)

E. Canaani	Guest Worker	LVD NIAID
C. Croce	Professor	Wistar Institute
F. Ruddle	Professor	Yale University
R. Weinberg	Professor	MIT

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Moloney sarcoma virus genome consists of two parts - a sarcoma specific portion of about 1,600 nucleotides located most likely near the 3' end of the MSV genome (∿900 bases from the 3' end) and in a single unique stretch - and a common part of 4,000-5,000 nucleotides located at the 5' region of the MSV genome and including a stretch of 600-900 nucleotides at the 3' far end of the genome. The sarcoma specific sequences can be found in a single copy on a discrete mouse chromosome (most likely 8, 11, or 12). The common sequences can be found in multiple poorly related copies (5-8) in mouse chromosomes but no attempt has been made yet to localize the involved mouse chromosomes. Work to localize the Moloney sarc integration sites in homologous and heterologous host DNA is currently in progress. In addition work to localize sarc on different cellular mRNA site classes is in progress.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To understand the structure and function of the Moloney sarcoma viral genome and the nature of the control of this genome in mammalian cells.

Methods Employed:

RNA-DNA and DNA-DNA hybridization assay hydroxyapatite chromatography and the Southern blotting technique. Cell DNAs prepared from mouse-human somatic cell hybrids. cDNA subsets prepared by the endogenous reverse transcriptase reaction and cycled by hybridization to viral RNAs and fractionation on hydroxyapatite.

Major Findings:

The Moloney sarc sequences are present in one closely matched copy in normal mouse DNA. The Moloney common sequences are not present in any closely matched copies in mouse DNA but have 3-8 copies of related sets of sequences in mouse DNA (Tm lower by 6°C). The sarc sequences are not expressed in normal mouse cell RNA or in chemically transformed mouse cell RNA. They are only expressed in MSV-transformed cell RNA. Hence, there may be multiple sets of "oncogenes" in mouse cells.

The sarc sequences in mouse cell DNA is highly conserved - in fact as conserved as the globin gene sequences in mammals. In contrast the common sequences are not conserved and no related sequences can be found in a closely related mouse species - Mus caroli. Hence, the different portions of MSV appear to have a distinct history, supporting the theory of a recent recombination event producing MSV. Further, the sarc sequences may have some important normal cellular function to be so highly conserved.

The finding of a few copies of MSV in mammalian cell DNA and its amplification with helper virus infection appears due to a specific interaction of MSV and helper virus in cells.

In collaboration with E. Canaani, we have found the majority of sarc sequences appear to hybridize with a Hinc II fragment located near the 3' end of MSV.

In collaboration with C. Croce and F. Ruddle the endogenous cellular sarc sequences segregate with mouse chromosomes 8, 11, or 12 in mouse-human hybrids. Work localizing the specific integration sites for MSV in mammalian cell DNA is currently in progress with R. Weinberg of MIT. Similarly, location of sarc sequences in different size classes of mRNA is currently being done with H. Fan, Salk Institute and L. Phillipson of MIT.

Significance to Biomedical Research and the Program of the Institute:

Recombination of cellular sequences with type C viral sequences appear to involve the same end of the type C viral genome. This may also be true of the leukemogenic recombinants - MCF and HIX (based on Lerner's results). Hence, DNA recombination events may be a prerequisite or activated post-script of transformation.

The site of recombination in the cellular DNA may be of importance in understanding the origins of mouse leukemia and sarcoma viruses. The finding of Fan and Phillipson of small viral mRNA's suggest processing and a distinct message for sarc translation.

While the significance of the different genes of MSV in producing disease is unknown, these studies in further localization of viral and cellular genes may aid in understanding their function.

Proposed Course:

Finish localization of sarc in provirus cell DNA, chromosomes and mRNA. Eventually, when conditions are adequately safe, cloning of the MSV fragments for eventual translation studies should be done.

Publications:

Frankel, A. E., Haapala, D. K., Neubauer, R. L. and Fischinger, P. J.: Elimination of the sarcoma genome from murine sarcoma virus transformed cat cells. Science 191: 1264-1266, 1976.

Frankel, A. E., Neubauer, R. L. and Fischinger, P. J.: Fractionation of DNA nucleotide transcripts from Moloney sarcoma virus and isolation of sarcoma virus specific complementary DNA. J. Virol. 18: 481-490, 1976.

Frankel, A. E. and Fischinger, P. J.: Nucleotide sequences in mouse DNA and RNA specific for Moloney sarcoma virus. Proc. Natl. Acad. Sci. USA 73: 3705-3709, 1976.

Frankel, A. E. and Fischinger, P. J.: Rate of divergence of cellular sequences homologous to segments of Moloney sarcoma virus. J. Virol. 21: 153-160, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04842-06-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Biochemistry and Biophysics of Leukemia and Sarcoma Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	L. A. Phillips	Microbiologist	LVC NCI
OTHER:	R. H. L. Pang	Visiting Fellow	LVC NCI
	P. J. Fischinger	Hd, Virus Control Section	LVC NCI
	A. E. Frankel	Senior Surgeon	LVC NCI
	D. K. Haapala	Microbiologist	LVC NCI
	S. Nomura	Microbiologist	LVC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project, as well as project number Z01-CP-04852-02-LVC, studies the biochemical and biophysical properties of nucleic acids and polypeptides of mammalian leukemia and sarcoma viruses. Our major findings for this fiscal year are as follows:

1. Poly(C)- and poly(G)-rich sequences are present in the RNA of leukemia and sarcoma viruses from avian, murine, feline, and primate isolates.
2. Little or no poly(C)- and poly(G)-rich sequences are present in the messenger RNA examined from normal mammalian cells.
3. Genomic and subunit RNA of mamalian leukemia and sarcoma viruses have regions of double-stranded RNA.
4. Gazdar murine sarcoma virus (Gz-MSV) and Moloney murine sarcoma virus (M-MSV) are closely related and possibly Gz-MSV is a variant of M-MSV as determined by nucleic acid hybridization studies and analysis of viral expression in cells.
5. These studies represent a possible molecular approach to cancer therapy for some types of viral neoplasia.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Our cancer research studies are concerned with the biochemical and biophysical properties of the nucleic acids and polypeptides of mammalian leukemia and sarcoma viruses. The ultimate goal of these studies is to make a molecular approach to cancer therapy for some types of viral neoplasia to become a reality. Specifically, the research encompasses the following areas:

1. The characterization of viral nucleic acids and polypeptides from purified retroviruses and from cells which are either infected with leukemia viruses or transformed by sarcoma viruses.

2. Partial sequence analysis of genomic and subunit viral RNA from leukemia and sarcoma viruses to determine if the viral RNA subunits are the same (polyploid) or different (haploid) in their nucleotide composition, excluding the poly(A)-, poly(C)-, and poly(G)-rich sequences.

3. The determination of the role of poly(A)-, poly(C)-, and poly(G)-rich sequences in the genomic and subunit viral RNA.

4. Studies to determine if the poly(C)- and poly(G)-rich sequences or tracts are transcribed into poly(dG)- and poly(dC)-rich tracts respectively in the viral DNA (cDNA) before it becomes intergrated into the cellular DNA.

5. The determination of the absence or presence of poly(C)- and poly(G)-rich sequences in the messenger RNA of nonmalignant, benign, and malignant human cells; after having detected little or no poly(C)- and poly(G)-rich tracts in the messenger RNA of mammalian cells.

6. Characterization of the double-stranded regions in the genomic and subunit RNA of mammalian leukemia and sarcoma viruses and a determination of their function.

7. The determination of the defectiveness of mammalian sarcoma viruses at the molecular level.

Methods Employed:

For characterization of the viral nucleic acids and polypeptides from retroviruses, standard biochemical and biophysical techniques (analytical and preparative ultracentrafugation, chromatography, nucleic acid hybridization, isotopic labeling, polyacrylamide gel electrophoresis, peptide and oligonucleotide fingerprinting, electrofocusing, *in vitro* protein synthesis, enzymology, etc.) are being employed.

Major Findings:

Some of the major findings resulting from these biochemical and biophysical studies on the nucleic acids of mammalian leukemia and sarcoma viruses, which have been published in major scientific journals and presented at both national and international meetings of note, are as follows:

1. The subunits RNA of mammalian sarcoma viruses is 28S to 30S in contrast to 30S to 40S RNA subunits for leukemia viruses.

2. The 3'-termini of viral RNA subunits from mammalian leukemia and sarcoma viruses contain poly(A)-rich sequences.

3. Poly(A)-, poly(C)-, and poly(G)-rich sequences are present in the RNA of leukemia and sarcoma viruses; however, poly(U)-rich tracts are absent in the viral RNA.

The detection of homopolyribonucleotide sequences in the RNA of leukemia and sarcoma viruses has not yet been confirmed by other scientists, possibly because of the following: (1) the complexity of the research, (2) the cost of doing the research, (3) the lack of necessary materials commercially, or (4) possibly the completeness of our homopolyribonucleotide tract studies, since we have shown that the poly(C)- and poly(G)-rich tracts are present in the RNA of retroviruses from avian to primate isolates. Because of the interest in our work by others, P-L Biochemicals have now made the necessary materials available commercially for the detection and isolation of the homopolyribonucleotide tracts. I am very excited and confident about the detection of the ribohomopolymeric tracts by means of polynucleotide agarose affinity chromatography because the specificity, sensitivity, and selectivity of complementary binding of homopolyribonucleotides to these affinity columns are superb. The specificity of binding is ideal as indicated by the fact that complementary binding to the columns exceeds 90% in comparison to noncomplementary binding of less than 3%. The sensitivity of binding studies indicate that a sequence of a minimum of 5 to 10 homoribonucleotides are required for complementary binding to occur to the polynucleotide agarose affinity columns. The selectivity of binding studies indicate that both synthetic alternating copolymers and native E. coli tRNA do not bind to the affinity columns, thus confirming the fact that for complementary binding to occur, a sequence or tract must consist of a minimum of 5 to 10 homoribonucleotides. Base composition analysis on the poly(C)-rich tracts from the RNA of retroviruses indicate that they are composed of approximately 70% CMP. Oligonucleotide fingerprinting to detect these tracts has not been done because of the excellent specificity, sensitivity, and selectivity of the polynucleotide agarose affinity columns employed and the subsequent chemical analysis of the isolated tracts. However, liquid chromatography studied are planned to better resolve and quantitate the nucleotides in the excised sequences. Also enzymatic studies with purified

exonucleases and heteroduplex studies with poly(dI), poly(dG), and poly(dC) and the RNA of leukemia and sarcoma viruses are planned in order to locate the position of the poly(C)- and poly(G)-rich tracts within the viral RNA.

Four classes of mammalian viral RNA subunits have been identified according to their homopolyribonucleotide tract composition and the subunits assembled into a proposed genomic model for mammalian retroviruses. The four classes of subunits are different (haploid) if the homopolyribonucleotide sequences are included in the nucleotide composition of the subunits; however, if these sequences are excluded in the nucleotide composition of the subunits, then we are unable to answer the question as to whether the viral RNA subunits are haploid or polyploid. There is no universally accepted model for the genome of mammalian RNA-tumor viruses at the present time and, in my opinion, this nonmonolithic view among scientists is healthy. Many models have been proposed by competent scientists. The main, unresolved question to everyones satisfaction, is the subunit composition of the genomic viral RNA. Bader of NCI has proposed a monomer model for the genomic RNA of retroviruses and believes that viral RNA subunits may be only configurational variants of the genomic molecule. While Davidson of Cal Tech has proposed that the genomic viral RNA is composed of two subunits that are either homodimers (SS, MM, and LL) or heterodimers (SM, SL, and ML). We have proposed that the genomic viral RNA is composed of four subunits according to their homopolynucleotide tract composition. A possible explanation that could possibly resolve the differences between Davidson's model and our model is that each of the dimers in Davidson's model could possibly consist of two subunits, thus making a total of four subunits per genomic RNA. The two subunits could be joined together by a 3' to 5' linkage within the loops that Davidson sees in his electron micrographs, since I do not believe that his laboratory has prepared individual subunits from the genomic viral RNA and compared their lengths to the lengths of the subunits observed in his dimer model. In our four subunit genomic model, each of the two subunits have been connected together by a 3' to 5' linkage by means of the poly(C)- and poly(G)-rich tracts. There is some agreement of an approximate 3×10^6 MW for the 30 to 40S subunits viral RNA and an approximate 10×10^6 MW for the 60 to 70S genomic viral RNA. The MW of the genomic viral RNA is incompatible with only two each 3×10^6 MW subunits. Ultimately, we would like to isolate sufficient amounts of each of the four types of subunits according to their homopolyribonucleotide tract composition and reassociate them *in vitro* into genomic viral RNA. Transfection studies with DNA, which have only been done with the viral DNA from a very limited number of mammalian retroviruses, most noticeably RD-114, would seem to possibly suggest that only one viral RNA subunit is required for infection. However, since transfection experiments have not been able to be carried out with viral RNA subunits themselves but with viral DNA of an approximately equivalent molecular weight, perhaps one should not arrive at this conclusion. In these transfection experiments, there could have been viral DNA containing poly(dC)-rich tracts and some containing poly(dG)-rich tracts, if the poly(C)- and poly(G)-rich tracts in the viral RNA are transcribed. The viral DNA used in the transfection studies was not fractionated by poly(C)- and poly(I)-

agarose affinity chromatography to determine if there were different species of viral DNA according to their homopolydeoxyribonucleotide tract composition. It is possible that in vivo recombination occurs between different types of viral DNA before it becomes infectious or alternatively the poly(dC)- or poly(dG)-rich tracts, if present, may not be required for infectivity. Further studies are required to understand the transfection phenomenon in relation to the viral RNA subunits composition of the genomic viral RNA.

So at the moment, there is no universally acceptible genomic model for mammalian retroviruses. Additional research is required to resolve unequivocally the number of subunits per genomic viral RNA. We hope to further answer this question by some of our current studies with isolated double-stranded RNA fragments from the genomic viral RNA. We are dissociating the dsRNA fragments to ssRNA fragments and determining the ½ Crt reassociation or renaturation values of the ssRNA fragments to both genomic and subunits viral RNA in hope of obtaining additional evidence of the number of subunits per genomic viral RNA.

Significance to Biomedical Research and the Program of the Institute:

A molecular approach to cancer therapy for some types of viral neoplasia has been proposed from our biocemical and biophysical studies on the structure and nucleotide composition of the RNA of mammalian leukemia and sarcoma viruses. Partial sequence analysis of the RNA of these viruses demonstrated the existence of poly(A)-, poly(C)-, and poly(G)-rich sequences. These sequences or tracts have been detected in the RNA of leukemia and sarcoma viruses from avian to primate isolates. Poly(A)-rich sequences have also been found in normal, nonviral messenger RNA of mammalian and human cells. We have shown that comparatively little or no poly(C)- and poly(G)-rich tracts exist in rabbit globin messenger RNA in comparison to the amount of these tracts in the RNA of retroviruses. If it can be determined definitively that the RNA of leukemia and sarcoma viruses contain specific homopolyribonucleotide tracts which are either absent or present minimumly in normal, nonviral messenger RNA of human cells, then it may be possible to make sequence or tract specific drugs against the unique viral specific sequences to selectively block viral nucleic acid functions at the transcriptional level while not inhibiting cellular messenger RNA functions. If this selective blocking of leukemia and sarcoma virus functions can be accomplished, a molecular approach to cancer therapy can become a reality for some types of viral neoplasia. However, the messenger RNAs of normal and benign human tissues have not been examined by me for the presence or absence of these homopolyribonucleotide tracts as the rabbit globin messenger RNA was examined, because of a lack of professional and technical assistance. When I am given sufficient technical and professional assitance, I shall examine all of the normal and benign human tissues that I have for the presence or absence of these tracts. However, this is a tremendous job. It is imperative that it be done by me or other competent molecular biologists so that if little or no poly(C) and poly(G)-rich tracts are detected, as

in the mammalian globin messenger RNA, attempts can be made by the pharmacological chemists to make a sequence specific drug to bind to the poly(C)-rich tracts in the retrovirus RNA or possibly the transcribed poly(dG)-rich tracts in intergrated viral DNA to specifically inhibit the transcription of the viral nucleic acids without interferring significantly with the normal cellular processes. Also synthetic polyribo- or polydeoxyriboguanylic acid, and polyribo- or polydeoxyriboinosinic acid, if they can penetrate the cancerous cells and are not degraded by the intracellular nucleases, should be tested in vitro and in vivo to determine if they can specifically block the transcription of either the intracellular intergrated and nonintergrated viral nucleic acids. Also currently there are a few chemotherapeutic agents, such as the relatively nontoxic actinomycin derivatives of Drs. Formica of the Medical College of Virginia (MCV) and Katz of Georgetown University, adriamycin, and kethoxal of Upjohn Company that may be promising since their mode of action appears to be on either the poly(C)- or poly(G)-rich tracts or both.

Therefore, a molecular approach to cancer therapy for some type of viral neoplasia, in our opinion, can become a reality especially for human leukemia and cancer of the breast. Although no complete infectious human retrovirus has been isolated from human leukemia and cancer of the breast, the footprints of these viruses have been found, namely reverse transcriptase in human leukemia while blood cells (Gallo, et al.) and in particles from human breast tissue (Spiegelman, et al.). Also proviral sequences of primate retroviruses have been detected by hybridization studies in some leukemic patients (Gallo, et al.). Therefore, it is of considerable importance that a molecular approach to cancer therapy for some types of viral neoplasia be continued and vigorously investigated. Many scientists of note have indicated to me that our findings and approach are interesting and that they look forward to seeing future developments.

Proposed Course:

These critical biochemical and biophysical studies of nucleic acids and polypeptides from mammalian and human putative leukemia and sarcoma viruses will be continued in order of their complexity and the availability of adequate technical and professional assistance.

Publications:

Phillips, L. A. and Pang, R. H. L.: The detection and characterization of polynucleotide sequences in the RNA of mammalian oncornaviruses. In Clemmesen J. and Yohn, D. S. (Eds.): Bibliotheca Haematologica No. 43. Basel, Switzerland, S. Karger AG, 1976, pp. 460-464.

Phillips, L. A. and Pang, R. H. L.: Poly(C)- and Poly(G)-rich tracts in the RNA of oncornaviruses. Nieburgs, H. E. (Ed.): Third International Symposium on Detection and Prevention of Cancer. New York, Marcel Dekker, Inc. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04843-02-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
A. Morphologic aspects of herpesviruses during the infectious cycle
B. Macrophage activation in transplantable mouse tumors exposed to immunomodulators

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Luetzeler	Visiting Scientist	LVC NCI
OTHERS:	U. I. Heine	Research Microbiologist	LVC NCI
	M. Chirigos	Assistant Laboratory Chief	LRTV NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Ultrastructural Studies Section

INSTITUTE AND LOCATION
Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A. A time study was undertaken combining morphological methods with tracer studies on the entry of herpes type viruses into cells and subsequent early intracellular events have been followed by electron microscopy. Tracer studies were utilized to follow the incorporation of viral DNA into preformed empty nucleo capsids upon removal of an inhibitor of DNA synthesis. Structural differences of the viral core of various herpesviruses could be visualized by using different stains and fixatives on virus infected and transformed cells.
B. Mice with subcutaneous transplanted tumors were treated 7 days later with a synthetic agent (pyran copolymer). Controls received 0.9% NaCl. In vitro and histopathologic studies on activated macrophages of the host and tumor biopsies revealed the important role of these cells against tumor tissue. The drug therapy resulted in (a) enhanced macrophage inhibition of tumor cell DNA synthesis, (b) heavy accummulation of macrophages and histiocytes at the tumor site and (c) association of histiocytes with tumor cells.

Project Description

Objectives:

A. To study the relationship of selected tumor viruses of the herpes type to their host cells in respect to early and late events of infection and to investigate differences in the fine structure of the envelope and the core of antigenic different strains of herpesviruses.

B. To study the association of macrophage activation with anti-tumor activity by synthetic and biologic agents.

Methods Employed:

A. Tissue culture techniques for virus propagation, biochemical methods for concentration and purification of labeled virus, transmission electron microscopy combined with high resolution autoradiography and selective stainings and embeddings.

B. Light microscopy of tumor biopsies.

Major Findings:

A. Contradicting morphological observations have been published recently in respect to the uptake of herpes type virus into the cell. Experiments with highly concentrated and labeled virus revealed in a time study new results in regard to virus approach, attachment and penetration. Tracer studies clarified the mode of virus transportation through the cytoplasm into the nucleus as well as the spatial relationship of viral progeny to parental DNA. Mean times of uptake, transportation and eclipse could be calculated.

Hydroxyurea, known as an inhibitor of DNA-synthesis of herpes type viruses, permits the synthesis of some viral proteins and the formation of intranuclear capsids with low electron density. Preliminary morphological results concerning the assembly of immature herpes type viruses upon removal of hydroxyurea have been published recently. New experiments are initiated in this laboratory employing tracer studies to follow up in detail the way of DNA incorporation into preformed capsids.

The fine structure of herpes type viruses is very complex; the appearance of the virions, especially the spatial arrangement of infective DNA inside the viral core, is highly dependent on the methods used for its visualization and varies greatly. Since it is known that some herpes viruses differ from each other morphologically in regard to the fine structure of their cores, it is of interest to compare in detail the structure of tumor producing herpes viruses with those previously investigated.

Herpes virus ateles, isolated from spider monkeys, replicates between 5-7 days in owl monkey kidney cells with typical cytopathic effect. Electron microscopic

studies revealed that the nucleocapsids contain an electron dense core and that the capsids are enveloped at the inner nuclear membrane. A time study combined with morphologic examination of the material may provide new knowledge in respect to the mode of uptake, assembly of nucleocapsids and the release of mature virions from the cells.

B. Pyran, a synthetic anti-tumor drug, has been proven to suppress the growth of various murine solid tumors. Its resistance against neoplasia has been attributed to the effect of pyran on the host's reticulo-endothelial system. A comparative histopathological study revealed an intense histocytic reaction in the connective tissue surrounding the primary tumors in mice receiving pyran as compared to controls. Macrophages and histiocytes migrated actively into the tumor and were often associated with healthy or necrobiotic tumor cells. Furthermore, activated macrophages were recovered from pyran treated animals which potently inhibited DNA synthesis and growth of tumor cells in vitro.

Significance to Biomedical Research and the Program of the Institute:

A. Herpes viruses are known to cause cancer in primates and lower animals and are candidates for certain human cancers. Clarification of the early event of viral infection at the cellular level is important for the understanding of virus-host cell interaction. The replication of viral DNA is one of the most important steps of the viral cycle towards the production of infectious progeny virus. The investigation upon hydroxyurea block is performed on tumor causing viruses in order to clarify further the way of DNA incorporation and enfolding into preformed nucleocapsids. The knowledge of the fine structure of the viral envelope and the arrangement of the DNA inside the core of virions will be helpful for the understanding of the interaction between virus and host cell and may enable us to distinguish between herpes type viruses of antigenically different genera. Herpes virus ateles, as one of the lately isolated viruses of the herpes type, has potentials as a transforming agent. It is known to transform marmoset lymphocytes and produce malignant lymphomas in marmoset monkeys. The virus was also found to transform adult human blood lymphocytes. Therefore, it is important to learn to understand the events leading to infection, the replicative cycle and the release of mature viral particles from transformed cells.

B. Pyran copolymer has shown to be very effective as an anti-tumor drug, since it induces host resistance to the growth of various tumors through hyperplasia and activation of the reticuloendothelial system. Pyran therapy resulted in (a) enhanced macrophage inhibition of tumor cell DNA synthesis in vitro, (b) heavy accummulation and infiltration of histocytes and macrophages at the tumor site and; (c) the association of histiocytes with degenerate appearing tumor cells. The cure rate attained when pyran was combined with remission inducing chemotherapy and its effect on modulating host immunological factors even in advanced tumor system, strongly support the potential use of pyran copolymer as an adjuvant to other conventional tumor treatment modalities.

Proposed Course:

A. Experiments to determine the pathway of viral DNA from cell entry to appearance of viral progeny in the nucleus will be expanded to clarify the following points: the uptake of viral particles into the cell, the interaction between virus and cell in the early stage of infection, the way of transportation of viral DNA into the nucleus, the intranuclear sites of replication and the spatial relationship of the viral progeny to the parental DNA. The blocking mechanism of DNA replication by hydroxyurea will further the understanding - by means of high resolution autoradiography - of the following points: the way as well as the moment of DNA incorporation into nucleocapsids with low electron density, the relationship of filamentous structures to nucleoproteins, the visualization of the structural arrangement of the DNA during the process of incorporation into empty capsids, and the maturation of infectious virus particles upon hydroxyurea block release. The composition of viral envelope and the structural differences of the viral core will be studied by combing different fixatives with certain electron dense stains on infected cells grown in vitro.

Herpes virus ateles is not yet studied in detail in respect to the replicative cycle, the modes of uptake, transportation, replication of parental DNA, and maturation as well as release of progeny virus from infected tissue cultures.

B. Pyran given intraperitoneal, produces a histiocytic reaction at the subcutaneous tumor site. Since pyran copolymer has been proven to be very effective against some of the transplantable tumors in mice, it will be of great interest to know if the drug can reduce also the number of metastatic lesions which develop after intravenous inoculation of tumor cells. The mode of macrophage activation needs further examination in order to clarify whether enhanced macrophage accummulation around metastatic lesions occurs in situ or if the macrophages are transported from different places of origin by means of blood or lymph circulation to the tumor site. One or the other way would have great impact on the future treatment by activated macrophages.

Publications:

Schultz, R. M., Papamatheakis, J. D., Luetzeler, J., Ruiz, P. and Chirigos, M.: Macrophage involvement in the protective effect of pyran copolymer against the Madison lung carcinoma (M109). Cancer Res. 37, 358-364, 1977.

Ablashi, D. V., Twardsik, D., Easton, J., Armstrong, G., Luetzeler, J. et al.: Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus cell systems. Europ. J. Cancer (In Press).

Krueger, J., Graw, R. G., Buja, L. M., Bull, M. J., Herzig, G. P. and Luetzeler, J.: Side effects of combined chemotherapy in patients with bone marrow transplantation; effect on heart, lung and liver (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04849-04-LVC

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on Oncogenic Herpesviruses of man and subhuman primates

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Dr. John M. Easton	Medical Director	LVC NCI
OTHER:	Dr. Paul H. Levine	Medical Officer	LVC NCI
	Dr. Dharam V. Ablashi	Microbiologist	LDTV NCI
	Mr. Gary Armstrong	Biology Technician	LRTV NCI
	Dr. Josef Luetzeler	Visiting Scientist	LVC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION

Division of Cancer Cause & Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project concerned itself mainly with the relationship of Epstein-Barr virus (EBV) to nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma. A Nasopharyngeal Carcinoma Registry was established in order to relate histologic subtype to prognosis, to EBV content of the tumors, and to serologic responses to EBV. A literature review concerning naturally occurring tumors of the mouth, throat, nose and paranasal sinuses in domestic animals revealed that canine squamous carcinoma, especially of the tonsil, would be a good naturally occurring model for NPC. Study of an epithelial somatic cell hybrid containing the EBV genome revealed that this cell line would aggressively form tumors in nude mice and that mice could regulate the EBV genome content of the tumors. Thus, this system is a good laboratory model for NPC. Study of chemicals used as hair dye components revealed that one of them could induce the production of EBV early antigen in Raji cells. A study of the response of common marmosets (Callithrix jacchus) to EBV revealed that no tumors were induced in this species. Serologic responses to EBV were variable; thus, common marmosets should be reserved for studies with other oncogenic viruses.

Project No. Z01-CP-04849-04-LVC

Project Description

Objectives:

1. To evaluate the relationship of Epstein-Barr virus to human cancer, with particular emphasis on nasopharyngeal carcinoma and Burkitt's lymphoma.

2. To utilize animal models for the study of the pathogenesis and control of herpes virus induced diseases.

Methods Employed:

1. A Nasopharyngeal Carcinoma Registry was established in collaboration with the Armed Forces Institute of Pathology in Washington, D.C. and the Mayo Clinic in Rochester, Minnesota. Slides of nasopharyngeal carcinomas were obtained and attempts were made to subcategorize the disease into various histologic types. Epidemiologic and laboratory information relevant to the patients in the registry was obtained.

As a model for the human studies, a literature review concerning naturally occurring tumors of the mouth and throat, nose and paranasal sinuses in domestic animals was undertaken.

2. Athymic nude mice were studied for their ability to carry tumors containing Epstein-Barr virus (EBV). Cell inocula included HR-1, a Burkitt lymphoblastoid cell line, D98, a HeLa variant, and D98/HR-1, an epithelial somatic cell hybrid. Tumors and cell lines were examined for their EBV genome control by DNA-DNA reassociation kinetics and cRNA hybridization. They were also examined for their content of EBV nuclear antigen (EBNA), viral capsid antigen and early antigen. Chromosome studies were also performed. An additional animal model, the common marmoset (Callithrix jacchus), was evaluated for the production of tumors by EBV. In this study, common marmosets were inoculated intraperitoneally with two different lots of EBV from B95-8 (marmoset cell line producing $\geq 10^4$ transforming units (TU); HK-LY28 (nasopharyngeal carcinoma lymphoblastoid cell line producing $\geq 10^2$ TU), and NAB (American Burkitt's lymphoma cell line producing 10^1 TU). Animals were followed for the evolution of any disease, and for the development of antibodies to EBV viral capsid and early antigens.

3. The in vitro effects of 4-nitro-o-phenylenediamine (N1), 2-nitro-p-phenylenediamine (N2), 2, 4, - diaminotoluene (T1), and 4-methoxy-m-phenylenediamine (T2) on EBV and Herpesvirus saimiri (HVS) producer and nonproducer cells were studied. The solubility of the compounds N1, N2, T1, and T2 was determined, as were their toxic concentrations for the cell lines employed. Treated cells were studied by transmission electron microscopy, and were also studied for their content of EBV and HVS early (EA) and late (VCA) antigens.

Major Findings:

1. A higher incidence of nasopharyngeal carcinoma in a younger age group was found in American patients entered into the Registry. This confirms previous reports, and is in contrast to observations in some parts of Asia. A significant number of black patients with NPC was noted, allowing the evaluation of racial as well as geographic factors in American NPC patients. A system of follow up was established to allow a comparison of survival data, immune reactivity to EBV, epidemiologic information and histologic subtypes. Hopefully, an agreement between participating pathologists will be reached on histologic subclassification.

Eighty-four percent of cases of oral and pharyngeal cancer were in dogs (C. R. Dorn et al, J. Am. Vet. Med. Assoc., in press); malignant neoplasia of the mouth and pharynx was the fourth most common form of cancer in both dogs and cats. Squamous cell carcinoma was the second most common form of oral and pharyngeal cancer in dogs and the most common form in cats and horses. Similarly, of 300 neoplasms involving the nasal cavity, three-quarters were in dogs (B. R. Madewell et al. Amer. J. Vet. Res. 37, 851, 1976), and squamous cell carcinoma was found to be the third most common form of cancer. The frequency of these cancers varied in different breeds of dogs, and there was no consistent anatomic difference between breeds at excess versus those not at risk. Except for canine oral papillomatosis, we found no studies dealing with a possible viral etiology for the domestic animal neoplasms discussed here.

The most frequently observed oral and pharyngeal neoplasm in dogs (site and type) was squamous carcinoma of the tonsil (D. Cohen et al., Am. J. Vet. Res. 25, 1776, 1964). An unusual predominance of males was recorded for tonsillar carcinoma.

2. Tumors induced by cell lines were histologically identical regardless of whether the tumors were induced by cells from the cell lines (original inoculum), from tumors induced by those cells, or from cell lines derived from tumors. D98/HR-1-induced tumors were undifferentiated carcinomas, D98 tumors were carcinomas, and tumors induced by HR-1 cells were poorly differentiated lymphomas histologically reminiscent of Burkitt's lymphoma after treatment.

D98/HR-1 cells induced tumors with greater rapidity than did the HR-1 parental cells and had the highest percentage of tumor "takes". Tumors induced by the D98 parental cells were smaller than those induced by cells of the other 2 lines. Thus, tumors induced by the D98/HR-1 cells were more aggressive than those of the 2 parental cell lines. The greater aggressiveness of the hybrid cells may be related to their association with EBV.

EBNA was present in over 90% of HR-1 cells, which were also 10-15% VCA positive at the time of inoculation. This is reflected in the hybridization data, which indicate that there were over 600 EBV genome equivalents per cell. Touch preparations showed that approximately 80% of the cells from HR-1 tumors were EBNA positive. This suggests that most of the cells in the tumor were derived from the inoculum of HR-1 cells, and not from the host. Hybridization data on tumor material, however, showed that the number of EBV genome equivalents was sharply reduced - to 19 per cell. Cells from the line derived from the HR-1 tumor were lymphoblastoid, and over 90% were EBNA positive. The level of EA- or VCA-positive cells was 1% to 2%. This was reflected in the number of EBV genome equivalents in these cells, which was 170. The modal number of chromosomes in HR-1 cells was 47; no mouse chromosomes were observed.

Over 90% of D98/HR-1 cells were EBNA positive, but EA and VCA were repressed. However, these cells were inducible by iododeoxyuridine (IUdR) to produce significant quantities of both EA and VCA. Approximately 80% of the cells in imprint preparations from tumors induced by D98/HR-1 cells contained EBNA; no EA- or VCA-positive cells were observed. However, the number of EBV genome equivalents in tumor cells was sharply reduced. There was some recovery of viral genomes in an early in vitro passage of a cell line derived from a tumor, but full recovery occurred only in a later passage. Cells from a line derived from a tumor induced by D98/HR-1 cells were fully inducible by IUdR to produce both EA and VCA. Preliminary data suggest that the chromosomes in these cells were all human and were comparable to the original D98/HR-1 cells. No evidence has been found for the presence of C-type particles in the D98/HR-1 cells.

We noted no clinical manifestations over a period of 6 months in the common marmosets which had received any of the 4 lots of EBV. Animals which had received one lot of B95 EBV developed antibodies both VCA and EA, to a level of 320 for the anti-VCA and 80 for the anti-EA. However, the pattern of response to these antigens in these 2 animals was not consistent. No antibodies to EBNA were detectable. The other lot of B95 EBV caused the induction of anti-VCA to a level of only 4; no antibody to EA was detectable. The number of transforming units of EBV in both lots was the same; this further emphasizes the lack of consistency in the response of common marmosets to EBV. Animals which had received EBV of HK-LY28 or NAB derivation did not develop detectable antibodies to EBV.

3. The T dyes were much more toxic to cells than the N dyes. At all dose levels of dye, treated human and simian cells were significantly enlarged and revealed activation and concentration of cell organelles (mitochondria, ribosomes, and Golgi apparatus). Only cells treated with N dyes showed chromosome aberrations and fragmentation with a low mitotic index. Dye T2 induced EBV EA in Raji cells but failed to cause the production of VCA or virus particles. No dye had any effect on EBV antigen or particle production in P3HR-1 cells. None of the dyes affected the production of HVS or HVS antigens.

Significance to Biomedical Research and the Program of the Institute:

1. EBV, a known human pathogen which induces infectious mononucleosis in man, is a strong candidate for being a human oncogenic virus. EBV has been documented to cause tumors in subhuman primates and evidence is accummulating that EBV is strongly associated with the etiology of Burkitt's lymphoma and nasopharyngeal carcinoma in man. The establishment of a Nasopharyngeal Carcinoma Registry will enable studies of the role of EBV in this disease to quicken. It will assist the many studies utilizing laboratory assays with this virus as adjuncts to the diagnosis and treatment of the human tumor. Since many studies in the treatment of EBV-related tumors cannot be performed in humans, several animal models need to be evaluated. The review of spontaneously occurring oral, pharyngeal, nasal and paranasal neoplasms in domestic animals is significant because it revealed that squamous carcinoma of the dog in these anatomic areas would serve as a useful model for human nasopharyngeal carcinoma. The canine neoplasms could be used in attempts to isolate viruses, to elucidate other factors possibly involved in tumor etiology, and to assay treatment with antitumor agents. The variation in frequency of these neoplasms in different breeds of dog may serve as a useful model for the variation in incidence of nasopharyngeal carcinoma in different human populations. Squamous carcinoma of the tonsil in the dog is particularly worthy of comment because lymphoepitheliomas occur in man both in the nasopharynx and in the tonsil. Thus, this canine neoplasm may be of particular use as a model for human nasopharyngeal carcinoma. The relative shortness of the life span of the dog, our ability to manipulate and sacrifice it if necessary, and the intimacy with which this species shares man's environment, makes the dog an excellent animal for comparative epidemiologic studies.

2. The study of the EBV-containing tumors in nude mice is significant for a variety of reasons. The tumors induced by D98/HR-1 and HR-1 cells may be useful and inexpensive models for studying the regulation of the EBV genome in epithelial and lymphoid cells. They may also be useful for the evaluation of anti-viral and anti-tumor drugs.

In HR-1 cells, most of the EBV DNA may be involved in the production of virus particles in a small fraction of cells, with a remaining fraction of about 10 genome equivalents or less in a stably associated latent form in each cell. The sharp reduction in the number of EBV genome equivalents in the HR-1 tumor cells may be explained by assuming that virus production was stopped *in vitro*. In the case of the hybrid cells, the EBV genomes are latent and stably associated with the host cell. Passage *in vitro* may therefore influence the number of EBV genomes in both the productive and nonproductive state. We do not know if the influence is in anyway related to residual cell-mediated or humoral immunity in nude mice. The reduction of the number of EBV genome equivalents is probably not due to the presence of a large percentage of nonmalignant mouse cells, since at least 80% of the cells in the touch preparations of the tumors were EBNA

positive. In contrast, the reduction of EBV DNA was 300 fold in HR-1 cells and approximately ten fold in D98/HR-1 cells. However, further work will be necessary to strengthen this conclusion, to rule out sampling bias in the selection of specimens for the detection of EBNA or EBV DNA.

The results with HR-1 cells in these experiments are reminiscent of the situation with Burkitt's lymphoma. In the patient, tumor cells may contain the EBV genome and EBNA; EA and VCA have not been demonstrated. However, genome-positive tumor cells cultured in vitro may spontaneously produce EA, VCA, and complete virus. The study of HR-1 cells in the nude mouse may shed some light as to why this phenomenon occurs.

Trumper et al. (Int. J. Cancer 17, 578, 1976) found that EBV could be activated in human nasopharyngeal carcinoma cells, which had been passaged in nude mice, by treatment with bromodeoxyuridine. However, these cultures grew well in vitro for only three weeks. The epithelial D98/HR-1 hybrid cells in these present experiments could also be activated to produce VCA, EA and virus particles by IUdR after passage in nude mice, but their life-span is potentially infinite. Thus, D98/RH-1 cells are an excellent model for the study of EBV in epithelial cells, since there are presently no established epithelial cell lines which have been derived from nasopharyngeal carcinomas.

The results with EBV in common marmosets are significant because they are in contrast to the results obtained by other investigators (L. Falk et al., Int. J. Cancer 17, 785, 1976), who found that EBV could induce a lympho-proliferative disease in this species. Because EBV has such a variable effect in common marmosets, this species should be reserved for experiments with other oncogenic viruses in light of the shortage of New World primates.

3. The activation of EBV EA in Raji cells by hair dye T2 is significant because this compound is in a product for human use in the area of the head and neck. EBV may be involved in the etiology of a tumor of the head and neck - nasopharyngeal carcinoma. The interaction of compound, cell, and virus points out the complexity of the relationship between virus and host cell. The elucidation of the relationship of EBV to the etiology of nasopharyngeal carcinoma will have to take into account the effects of other factors, such as chemicals or other environmental agents, which may act as co-carcinogens or as enhancers.

Proposed Course:

Primary emphasis will be placed on obtaining additional cases for the American Nasopharyngeal Carcinoma Registry. Histologic features will be related to epidemiologic and survival data to improve the characterization of this disease and facilitate the identification of etiologic factors. Patients who agree to undergo laboratory evaluation will be studied for immunity to EBV, and the tumors will be tested for the presence of EBV genome. Other head and neck cancers will be used as controls.

In collaboration with Mr. Roger Connelly, an unselected group of patients will be evaluated by the Third National Cancer Survey and follow up studies for additional data relevant to the etiology of NPC. In conjunction with Dr. Paul Levine, comparable studies will be continued as in the past utilizing the American Burkitt Lymphoma Registry.

Publications:

Ablashi, D. V., Easton, J. M. and Aaslestad, H. G.: Advances in research on oncogenic herpesviruses. In: Proceedings of the Official Opening, Consultation and International Workshop on Advances in Comparative Virology. Report No. 1 of the WHO Collaborating Center for Collection and Evaluation of Data on Comparative Virology. Munich, UNI-Druck, 1976, pp. 291-326.

Ablashi, D. V., Easton, J. M. and Guegan, J. H.: Herpesviruses and neoplasia in man and subhuman primates. Biomedicine 24: 286-305, 1976.

Ablashi, D. V., Armstrong, G. R. and Easton, J. M.: Absence of horizontal transmission of Herpesvirus saimiri between experimentally infected and non-infected owl monkeys. Cancer Letters 2: 35-40, 1976.

Twardzik, D. R., Ablashi, D. V., Easton, J. M. and Armstrong, G. R.: Soluble complement-fixing antigens in Epstein-Barr virus cell systems. Bibl. Haematol. 43: 412-416, 1976.

Ablashi, D. V., Armstrong, G. R., Fellows, C., Easton, J. M. and Twardzik, D.: Evaluation of the effects of phosphonoacetic acid (PAA) and 2-deoxy-d-glucose (2DG) on Herpesvirus saimiri (HSV) in Vero cells. In: Third International Symposium on Detection and Prevention of Cancer, New York, 1976 (In Press).

Ablashi, D. V., Easton, J. M. and Armstrong, G. R.: Oncogenic DNA viruses: Experimental animals and in vitro systems for propagation, assay, transformation and tumor induction. In: Proc. Int. Symp. Exptl. Animals and In Vitro Systems in Med. Microbiol., Munich, 1976 (In Press).

Ablashi, D. V., Twardzik, D. R., Easton, J. M., Armstrong, G. R., Luetzeler, J., Jasmin, C. and Chermann, J-C.: Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus-cell systems. Europ. J. Cancer (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04850-05-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Reversion of Mouse Cells Transformed by MSV

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Nomura	Microbiologist	LVC NCI
OTHER:	P. J. Fischinger	Hd, Virus Control Section	LVC NCI
	R. H. Pang	Visiting Fellow	LVC NCI
	J. W. Hartley	Research Microbiologist	LVD NIAID
	C.F.T. Mattern	Microbiologist	LVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Moloney (M)-MSV genome was present in the cell RNA of four cloned revertants derived from M-MSV-transformed S+L- M-3T3FL cells. However, MSV was not rescuable from these clones by MuLV, nor inducible by 5-iododeoxyuridine.

Clones of SC-1 cells were isolated following transformation by MSV-Z. They were characterized as S+L- cells, and the RNA from these cells hybridized with the complementary DNA from M-MSV.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

A. To investigate the mechanisms of cell transformation and reversion of the malignant process.

B. To characterize a transforming virus (MSV-Z) isolated from S+L- Moloney-3T3FL cells.

Methods Employed:

A. Biochemical and biophysical procedures such as isotopic labeling, isolation of nucleic acids, analytical and preparative ultracentrifugation, reverse transcriptase assays, preparation and characterization of cDNA and nucleic hybridization were employed.

B. Standard virological, biochemical and biophysical techniques were used. These include virus assay, cloning of cells, chemical induction, density gradient centrifugation, ^{3}H-uridine incorporation, reverse transcriptase assay and electron microscopy.

Major Findings:

A. Previous reports have described the biological characteristics of flat varients (revertants) from S+L- M-3T3FL cells. M-MSV could not be rescued from these revertants by superinfection with MuLV, nor induced by treatment with 5-iododeoxyuridine. To further investigate the genetic nature of reversion, we measured virus specific RNA in parental S+L- cells and in revertant clones. We found sequences homologous with M-MSV RNA not only in RNA from S+L- cells, but also in RNA from all revertant cells tested to various degrees.

B. We have previously reported a transforming virus (MSV-Z) from S+L-M-3T3FL cells. This virus is replication defective and formed in the absence of detectable murine helper virus. The envelope of this virus is distinct from the previously described murine C-type oncornaviruses based on glycoprotein interference and neutralization tests. To investigate the RNA of this virus, clonal SC-1 cell lines transformed by MSV-Z were isolated. These morphologically altered cells were characterized as S+L- cells biologically and physio-chemically, and the RNA in these cells demonstrated homology with the cDNA from M-MSV.

Significance of Biomedical Research and the Program of the Institute:

A. Studies of the virus expression at both the transcriptional and translational level will increase our understanding of the mechanism of viral oncogenesis and basic cellular regulatory controls.

B. Further immunological and biochemical studies of MSV-Z to understand the structure and function of MSV-Z, and the nature of the control of this genome in mammalian cells.

Publications:

Nomura, S., Fischinger, P. J., Hartley, J. W. and Mattern, C. F. T.: Induction of a novel transforming virus (MSV-Z) from sarcoma positive leukemia negative 3T3FL mouse cells. Virology 69: 635-646, 1976.

Nomura, S. and Fischinger, P. J.: Rapid screening assay for revertants of MSV-transformed cells. In Prescott, D. M. (Ed.): Methods in Cell Biology. New York, Academic Press, Inc., 1976, Vol. 14, pp. 229-236.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04851-02-LVC

PERIOD COVERED

July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies on the Pathogenesis and Treatment of Burkitt's Lymphoma in Ghana

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. J. Biggar	Senior Surgeon	LVC NCI
OTHER:	I. V. Perkins	Microbiologist	LVC NCI
	P. H. Levine	Medical Officer	LVC NCI
	J. Whang-Peng	Medical Director	DCT NCI
	I. Magrath	Visiting Scientist	DCT NCI

COOPERATING UNITS (if any)

P. Gerber, Bureau of Biologics, FDA, Health, Education and Welfare, Bethesda, Md. F. K. Nkrumah, Univ. of Ghana Med. School; E. C. Christian, Univ. of Ghana Med. School, Accra, and W. Henle, Children's Hosp., Philadelphia

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION

Office of the Chief

INSTITUTE AND LOCATION

Division of Cancer Cause and Prevention, NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Burkitt's lymphoma (BL) is of great importance to human tumor studies because of the epidemiologic, virologic and immunologic evidence that it is virus-induced. The Burkitt Tumor Project (BTP) in Accra, Ghana, is an attempt to systematically study a series of BL patients in order to clarify the pathogenesis of the disease and develop better methods of control. Current studies are evaluating the possibility of different strains of Epstein-Barr Virus (EBV) as the cause of BL, looking at humoral and cell-mediated immunity to EBV and tumor-associated antigens as diagnostic and prognostic tools in BL, and investigating the role of malaria as a cofactor in disease etiology.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To develop virologic, immunologic and epidemiologic studies to determine whether Epstein-Barr Virus is etiologically related to Burkitt's lymphoma and whether antibodies can be used as a diagnostic or prognostic tool.

2. To determine the role of cellular immunity in Burkitt's lymphoma with particular attention to the pattern of delayed hypersensitivity to antigens derived from cultured Burkitt's lymphoma cells.

3. To study the role of malaria in Burkitt's lymphoma.

4. To study the clinical features of Burkitt's lymphoma in order to evaluate treatment regimes, clinical features of the disease, and factors affecting prognosis.

Methods Employed:

Ghanaian patients suspected of having Burkitt's lymphoma were referred by government and mission hospitals from all parts of Ghana. The majority of the patients came primarily from the southern third of Ghana, facility of transportation, population distribution and cultural considerations influencing this apparent greater number of cases from the south. Upon arrival in Accra, the patients were given a complete clinical work-up, including biopsy for histological examination and diagnosis. Pre- and post-treatment serum samples were collected at 1, 2, 3, 6, 9, 12, 18, and 24 months after diagnosis. These were matched with a single serum collection from asymptomatic controls, matched by age, sex and tribe. These sera were tested for antibodies to EBV viral capsid antigen and to the diffuse (D) and restricted (R) components of the early antigen complex (EA) by indirect immunofluorescence procedures. Antibodies to EBV-determined nuclear antigen (EBNA) were determined by the anti-complement immunofluorescence method. Patients with histologically confirmed BL were studied for cutaneous hypersensitivity to antigens derived from the Raji line of BL cells, to antigen from a normal cell line, to mumps, PPD and candida skin-test antigens. Tests were made pre- and post-treatment at intervals of 1 to 3 months post-treatment and at time of recurrent tumor.

Patients with histologically confirmed Burkitt's lymphoma were studied for factors which might influence prognosis, such as site(s) of original tumor, tumor burden and clinical stage of disease on presentation. Patients who had recurrent tumor within 12 weeks of attaining complete remission were considered to have early relapse and those relapsing after 12 weeks were classified as having late relapse.

Throat washings were obtained by gargling with medium RPMI 1640 followed by the addition to the gargle of 5% fetal calf serum and storage at minus 70 degrees C. Chromosome preparations were made from tumor biopsies by standard methods.

Major Findings:

Skin testing of Burkitt lymphoma patients with membrane extracts from a Burkitt derived cell line, Raji, indicated that patients with large tumor loads were less likely to respond to skin test. Similar findings were also observed with standard recall antigens. There was not a complete correlation between skin test reactivity between recall antigens and Raji extracts. A comparison of skin testing from the patients in Ghana and the patients in Hong Kong, Tunisia and the United States indicated that the Raji antigen was more likely to cause reactivity in lymphoma patients than cancer patients with other types of malignancy.

Virologic studies from throat washings and tumor specimens indicated that the EBV isolates obtained from Africans differed in biological and biochemical features from isolates obtained from other sources. For example, cord blood lymphocytes transformed by American EBV expressed only early viral functions (EBV nuclear and soluble complement-fixing antigens) and produced no detectable transforming activity. By contrast, cord-blood lymphocytes transformed by African EBV strains contained 0.2--0.3% of cells with EBV capsid and early antigen and produced EBV with transforming activity. Thus, the African isolates of EBV appeared to be biologically different than the isolates obtained in the United States.

Significance to Biomedical Research and the Program of the Institute:

Burkitt's lymphoma is one of the most important human tumors to be investigated because of the number of features indicative of a viral etiology. Seasonal variation in disease incidence, time-space clustering and confinement to areas of holoendemic malaria have emphasized the importance of environmental factors in the etiology of BL, and the recent detection of EBV genome in fresh BL tumor cells has strengthened the likelihood that this particular herpes virus is an important causal factor. The studies in this project are important in defining the relationship of EBV to BL through the integration of virological, epidemiological, immunological and clinical studies.

Proposed Course:

EBV isolates BL tumors from throat washing of BL patients, and from controls will continue to be studied for evidence of different biological properties with particular attention to comparison with isolates from NPC and IM patients.

Skin test reactivity will continue to be evaluated as a prognostic tool, and the results will be compared with new serologic and cell mediated immunity assays related to EBV.

Parameters relating to EBV infection and immunity will be compared in children infected heavily with malaria and children with a light burden to investigate the effect of malaria on the immune system and EBV immunity. The longitudinal study on EBV infection in newborn children and the evaluation of chromosomes in BL biopsies will continue.

Publications:

Levine, P. H., Ho, J., Nkrumah, F. and Biggar, R.: An evaluation of delayed hypersensitivity reactions to lymphoid cell lines and antibodies to Epstein-Barr virus. Oncogenesis and Herpesviruses III (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04852-02-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemistry, Biophysics, and In Vivo Expression of Mammalian Oncornaviruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. H. L. Pang	Visiting Fellow	LVC NCI
OTHER:	L. A. Phillips	Microbiologist	LVC NCI
	P. J. Fischinger	Hd, Virus Control Section	LVC NCI
	A. E. Frankel	Senior Surgeon	LVC NCI
	D. K. Haapala	Microbiologist	LVC NCI
	S. Nomura	Microbiologist	LVC NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Virus Control Section

INSTITUTE AND LOCATION Division of Cancer Causé & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project as well as project number Z01-CP-04842-06-LVC, studies the biochemical and biophysical properties of nucleic acids of mammalian oncornaviruses. A summary of our major findings for this fiscal year are as follows:

1. Poly(C)-rich and poly(G)-regions were detected in avian, feline, murine and primate leukemia and/or sarcoma viruses.
2. Gazdar murine sarcoma virus (Gz-MSV) is closely related to Moloney murine sarcoma virus (M-MSV) and Gz-MSV is possibly a variant of M-MSV.
3. The MSV-specific region in both Gz-MSV and M-MSV shared homology with hamster leukemia virus from F-2833 cells as determined by hybridization studies.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

The objectives of the proposed studies include:

1. Characterization of nucleic acids isolated from purified virions.

2. Isolation and characterization of viral specific sequences in producer and nonproducer cell lines after effective infection.

3. Determination of the role of adenylic acid-rich (poly(A)-rich) cytilylic acid-rich (poly(C)-rich) and guanylic acid rich (poly(G)-rich) sequences in genomic viral RNA and viral RNA subunits.

4. Studies on host modification of mammalian sarcoma viral genome in different cell lines and the effect of host modification on expression of viral sequences in the cells.

5. Studies on viral expression during the development and subsequent regression of virus induced mammalian tumors.

6. Studies on selective transcription of viral specific sequences and correlation of sequences transcribed to viral protein expression in cells.

Methods Employed:

Viruses were titered by either focus or helper assays. Characterization of nucleic acids involved analytical and preparative ultracentrifugation, affinity chromatography, hybridization isotopic labelling, polyacrylamide gel electrophoresis, etc.

Major Findings:

The Gazdar murine sarcoma virus (Gz-MSV) was characterized by nucleic acid hybridization and analysis of viral expression in cells. Gz-MSV is closely related to Moloney-MSV. The complete MSV-specific nucleic acid sequences constituted a major portion of Gz-MSV specific sequences. The MSV-specific sequences in both Gz-MSV and M-MSV genomes shared homology with HaLV nucleic acid sequences. Both rat cells (S+L-) and hamster (S+L-), infected with infectious or defective Gz-MSV expressed two viral proteins of M.W. 68,000 and 70,000 daltons. These proteins were immunologically related to p60 purified from ml isolates of M-MSV.

Significance to Biomedical Research and the Program of the Institute:

The biochemical and biophysical studies on the structure and nucleotide composition of RNA of mammalian leukemia and sarcoma viruses, coupled with analysis of viral specific sequences in cells after effective infection, could facilitate better understanding of the regulation of phenotypic expression of specific viral sequences, ranging from the presence of viral specific antigenic determinants to production of defective or infectious viral particles. The addition, deletion, and/or amplification of all or part of viral sequences in cells could be utilized to correlate specific viral sequences to specific phenotypic expression. The analysis of the functional sequences in detail could facilitate effective chemotherapy of neoplasia.

Proposed Course:

These critical biochemical and biophysical studies of nucleic acids and polypeptides from RNA tumor viruses will be continued in order of their complexity.

Publications:

Phillips, L. A. and Pang, R. H. L.: The detection and characterization of polynucleotide sequences in the RNA of mammalian oncornaviruses. In Clemmesen, J. (Ed.): Bibliotheca Haematologica, No. 43. Basel, Switzerland, S. Karger AG, 1976. pp. 460-464.

Phillips, L. A. and Pang, R. H. L.: Poly(C)- and poly(G)-rich tracts in the RNA of oncornaviruses. In Neeburgs, H. E. (Ed.) Third International Symposium on Detection and Prevention of Cancer. New York, Marcel Dekka, Inc. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04853-05-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Isolation and Characterization of Mammalian Type C Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. E. Benveniste	Microbiologist	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	C. J. Sherr	Medical Officer	LVC, NCI
	R. Callahan	Staff Fellow	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Different retroviruses have been isolated from various primate species by co-cultivation of tissues with heterologous cells. These isolates include type C viruses from several species of baboons (Papio) and from the closely related genus, Theropithecus. In addition, a group D retrovirus has been isolated from a langur monkey (Presbytis) cell line. This isolate is related to Mason-Pfizer virus (MPMV), but can be readily distinguished from MPMV by its host range, antigenic properties, and by nucleic acid hybridization. Various isolates from squirrel monkey (Saimiri) cell lines have also been obtained. These latter viruses are endogenous to squirrel monkeys, and are present in multiple copies in the cellular DNA of all squirrel monkey cells and tissues.

Three new retroviruses have been isolated from lung and spleen cells of the Southeast Asian mouse, Mus cervicolor. All three viruses are endogenous to this rodent species, and related nucleic acid sequences can be detected in the DNA of other rodent species. Two of the isolates, designated CERV C I and CERV C II, are typical type C viruses, while the third isolate shares certain morphologic and biochemical properties with murine type B viruses.

Project Description

Objectives:

To isolate type C viruses from various mammalian species and to examine the relationships among these viruses as well as their biological and biochemical properties.

Methods Employed:

Type C viruses are isolated by cocultivation of cell lines and tissues from various mammalian species with a wide variety of heterologous cell lines. The presence of a type C virus replicating in a cell line is usually first detected by assaying the pellet obtained after high speed centrifugation of supernatant fluid from cells with synthetic templates that can detect low levels of reverse transcriptase activity. Specific antisera prepared against all the known mammalian type C viral reverse transcriptases and radioimmunoassays for various type C viral proteins are employed to characterize new isolates. Radioactively-labeled ^{3}H-DNA copies of the various type C viral genomes are available to examine for the presence of related nucleic acid sequences in cell lines and tissues of various species and to characterize the species of origin of new isolates. A wide variety of virologic assays are also used to study the biologic properties of new type C isolates, such as host range, viral interference patterns, and presence of transforming (sarcoma) viruses.

Major Findings:

A. Isolation and characterization of primate retroviruses.

Retroviruses have been isolated from several different baboon species (genus Papio), from the closely related genus Theropithecus, from the langur monkey (Presbytis), and from squirrel monkeys (Saimiri). These viruses have all been isolated by cocultivation of primary cell lines derived from these animals with suitable heterologous mammalian cells.

Recent advances in the characterization of the reverse transcriptase reaction have allowed the generation, in vitro, of representative radioactively-labeled DNA copies of the viral genome. Fully representative DNA probes of the baboon, langur, Mason-Pfizer monkey virus, and squirrel monkey virus genomes have been prepared. Data obtained with fully complementary DNA transcripts of the baboon viral genome have confirmed earlier hybridization data. Within the primates, type C viral genes have evolved as the species have evolved, with virogenes from more closely related genera and families having more sequence homology than those from distantly related taxons. A comparison of the rates of evolution of viral gene sequences and that of other cellular sequences distinguishes these primates that have evolved in Africa from those that have evolved in Asia.

Similar nucleic acid hybridization studies have also shown that the squirrel monkey viruses are endogenous to squirrel monkeys, and are present in multiple copies in the cellular DNA of all tissues and cells of that species.

The cocultivation of a lung cell lines from the spectacled langur (Presbytis obscurus) with bat lung cells has resulted in the isolation of a new primate virus, designated PO-1-Lu. While PO-1-Lu is morphologically similar to MPMV, further characterization of the new isolate shows that it can be readily distinguished from MPMV by its host range, antigenic properties, and pattern of nucleic acid hybridization. The isolation of a virus related to MPMV from an Asian primate species whose natural habitat overlaps that of rhesus monkeys suggests that viruses similar to MPMV and PO-1-Lu may be naturally prevalent in at least certain Southeast Asian primate species.

B. Isolation and characterization of three distinct retroviruses from the Southeast Asian rodent, Mus cervicolor.

The cocultivation of a lung cell line from the Southeast Asian mouse Mus cervicolor with cells from heterologous species has resulted in the isolation of two new distinct type C viruses. Both viruses are endogenous to Mus cervicolor and are present in multiple copies in the cellular DNA of these mice. One of the viruses, designated M. cervicolor type C I (CERV C I), replicates readily in the SIRC rabbit cell line and is antigenically related to the infectious primate type C viruses isolated from a woolly monkey (SSAV) and gibbon apes (GALV). This virus is also closely related by both immunologic and nucleic acid hybridization criteria to a type C virus previously isolated from a second Asian murine species, Mus caroli. The isolation of the CERV C I virus thus provides further evidence that the infectious primate type C viruses originated by trans-species infection of primates by an endogenous virus of mice. The second virus, designated M. cervicolor type C II (CERV C II), replicates well in various cell lines derived from the laboratory mouse, Mus musculus. While antigenically related to type C viruses derived from M. musculus, the CERV C II isolate can be readily distinguished from standard murine leukemia viruses. An examination of the cellular DNA of other rodent species for nucleic acid sequences related to the genome of both CERV C I and CERV C II reveals that both viruses have been highly conserved evolutionarily, and that other species of rodents, such as laboratory mice and rats, contain endogenous virogenes related to those in the DNA of M. cervicolor.

The cocultivation of spleen cells from M. cervicolor with cells of the laboratory mouse (M. musculus) has also resulted in the isolation of a retrovirus designated M432. ^{3}H-DNA transcripts of the viral RNA genome of M432 detect mutliple copies of endogenous virogene sequences in the cellular DNA of normal M. cervicolor. Partially related nucleic acid sequences are also detected in the DNA of M. caroli and M. musculus as well as in more distantly related species (rat and hamster), reflecting the evolutionary conservation of these gene sequences in rodents. Although this retrovirus from M. cervicolor shares certain morphologic and biochemical properties with murine type B viruses, the new isolate is unrelated by nucleic acid hybridization

criteria to the mouse mammary tumor virus or known murine type C viruses, including the two endogenous type C viruses isolated from Mus cervicolor. The DNA of M. cervicolor therefore contains multiple copies of at least three distinct classes of endogenous viral genes.

Significance to Biomedical Research and the Program of the Institute:

The cocultivation techniques which have resulted in the isolation of endogenous primate type C viruses from baboons should be applicable to the isolation of a variety of mammalian type C viruses.

We have shown that all higher primates, including man, contain information in their tissues (antigens and nucleic acid sequences) related to endogenous primate type C viruses.

The discovery that many of the mammalian type C viruses arose by trans-species infection shows that viruses of one species can readily infect another species in vivo. For example, the infectious primate type C viruses (isolated from gibbon apes and a woolly monkey) most likely represent a very recent, perhaps contemporary, infection with viruses from a Southeast Asian rodent. These viruses have not yet been incorporated into the genomes of all members of the infected species. As such, these viruses appear readily capable of producing neoplastic disease in primates.

Proposed Course:

We are attempting to isolate new type C viruses, especially from primate and human tissues. Using ^{3}H-DNA probes prepared from all known mammalian type C viruses, we are attempting to find nucleic acid sequences in a variety of human tumors related to one of these type C viruses.

The apparent generality of trans-species infection of type C viruses raises the possibility that these viruses may cause neoplasia when they are in the process of being transferred from one species to another. Since there are many closely related animals available that either possess or lack virogene sequences, this possibility is being actively investigated by inoculating the appropriate animals with type C viruses.

Publications:

Todaro, G.J., Sherr, C.J. and Benveniste, R.E.: Baboons and their close relatives are unusual among primates in their ability to release nondefective endogenous type C viruses. Virology 72: 278-282, 1976.

Benveniste, R.E. and Todaro, G.J.: Evolution of type C viral Genes: Evidence for an Asian origin of man. Nature 261: 101-108, 1976.

Sherr, C.J., Benveniste, R.E., Lieber, M.M. and Todaro, G.J.: Type C viruses from Kirsten sarcoma-transformed mink cells co-cultivated with primate cells and expressing p30 antigens related to feline leukemia virus. J. Virol. 19: 346-358, 1976.

Callahan, R., Benveniste, R.E., Sherr, C.J., Schidlovsky, G. and Todaro, G.J.: A new class of genetically transmitted retravirus isolated from Mus cervicolor. Proc. Natl. Acad. Sci. USA 73: 3579-3583, 1976.

Benveniste, R.E., Callahan, R., Sherr, C.J., Chapman, V. and Todaro, G.J.: Two distinct endogenous type C viruses isolated from the Asian rodent Mus cervicolor: Conservation of virogene sequences in related rodent species. J. Virol. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04855-02-LVC

PERIOD COVERED
July 1, 1976 to December 31, 1976

TITLE OF PROJECT (80 characters or less)

Studies on the Antigens of Virus-transformed Leukemia Cells

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. H. Chen	Staff Fellow	LVC NCI
Other:	T. Aoki	Head, Immunology Section	LVC NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Carcinogenesis

SECTION
Immunology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Identification of cell surface antigens of AKR spontaneous leukemia by combination of double immunoprecipitates and electrogelphotograms.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

To characterize the properties of surface antigens on AKR spontaneous leukemia cells.

Method Employed:

Cells used are AKR spontaneous leukemia cells and normal AKR lymphoid cells. Cells were labelled with ^{125}I or ^{3}H-glucosamine. The membrane was then solubilized and precipitated by antisera prepared against leukemia cells and viral components. The immune precipitate was then analyzed by polyacrylamide gel electrophoresis.

Major Findings:

The electropherogram of the immunoprecipitate formed by the preabsorbed (C57BL/6 x C3Hf/He)F1 anti-AKR spontaneous leukemia (KSL) cell serum and ^{125}I-labelled KSL-cell lysate showed that the surface of KSL cell contained gp75 in addition to known MuLV structural protein gp70, p30, and p15(E). This gp75 shared antigenic determinants with p30 and p15(E). AKR virus infected nonmalignant cells such as thymocytes from normal six-week-old AKR mice or AKR virus-infected SC-1 cells did not contain gp75.

Significance to Biomedical Research and the Program of the Institute:

The possession of antigenic determinants of p30 and p15 on gp75 molecule suggests that with respect to the antigenic properties gp75 was very similar to the precursor proteins of mammalian type C RNA viruses Pr 65-70 with the difference that gp75 was glycosulated. It is of great interest that AKR spontaneous leukemia-specific cell-surface antigen acquired by malignant transformation was related to the MuLV structural proteins p30 and p15(E). These findings quite nicely correspond to the previous results obtained by immunofluorescence microscopy (IFM), bridging between classical and molecular immunologies. In other words, several lines of transplantable KSL have been established and the specificities of their cell surface antigens have been determined by IFM. Some lines have individual specific surface antigens in addition to the common cell-surface specific antigens. These antigens appear to be acquired by malignant transformation and can be distinguished from MuLV-associated surface antigens on nontransformed cells. The present results obtained by immunochemical approach correspond to those obtianed by IFM at molecular level.

Proposed Course:

To purify gp75 from the surface of AKR spontaneous leukemia cells for the determination of malignant cell specific antigen determinants.

To apply the same approach to other systems such as Moloney MuLV-induced leukemia system, gibbon ape leukemia system, and human tumor system.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04864-05-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analysis of Type C Virus-associated Surface Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. Aoki	Head, Immunology Section	LVC NCI
Other:	P. B. Brandchaft	Staff Fellow	LVC NCI
	J. H. Chen	Staff Fellow	LVC NCI
	H. Kimura	Visiting Fellow	LVC NCI
	S. Suzuki	Guest Worker	LVC NCI

COOPERATING UNITS (if any)

M. Liu	Chemist	Litton Bionetics Laboratory, Inc.
S. J. deKozlowski	Biol. Lab. Tech.	Litton Bionetics Laboratory, Inc.

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Immunology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To establish specificities of naturally occurring antibodies in sera from healthy humans, and healthy gibbon apes against envelope antigens of type C viruses from primates.

Examination of enhancing and inhibiting factors of virus-induced primary tumors from blood platelets.

Alteration of specificity of type C virus-associated surface antigens after transplantation or culture passages of mouse malignant cells.

Bridging between classical and molecular immunologies.

Model experiments of immunotherapy of AKR spontaneous leukemias using a preparation of Streptococcus pyogenes.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To analyze the specificity of naturally occurring antibody in primates to antigens on the envelope of type C virus isolated from mammalians.

2. To determine the role of blood platelets in the growth of type C virus-induced tumors.

3. To examine the alteration of type C virus-associated surface antigens after long-term transplantation or many culture passages of mouse malignant cells.

4. To analyze further the AKR spontaneous leukemia-specific surface antigens by combination of double immunoprecipitates and electrogelphotograms using the results obtained by immunofluorescene microscopy as a pilot.

5. To study the effect of lyophilized Streptococcus pyogenes on the cumulative mortality of AKR mice from spontaneous leukemias and on the host immune response.

Methods Employed:

1. Immunoelectron microscopy (IEM) and absorption of test sera with various cells.

2. Purification of platelets, homogenization, fractionation of soluble components, and inoculation of them with type C virus into mice or chicken.

3. IEM, ^{51}Cr-releasing lymphocyte-mediated cytotoxicity tests, and XC tests.

4. SDS polyacrylamide gel electrophoresis, double immunoprecipitation using radioisotope-labelled poly peptides, and immunofluorescence microscopy using viable cells.

5. Long-term inoculation with a coccus preparation, examination of humoral antibody formation, detection of reverse transcriptase in the plasma, and demonstration of interferon production.

Major Findings:

1. Positive high titer human sera contained natural antibodies to interspecies- or group-specific antigens on the envelope of the following type C viruses isolated from primates: gibbon ape leukemia virus (GALV), simian sarcoma virus, baboon endogenous virus, putative human virus isolated from a patient with acute myelogenous leukemia (HL23V) and from human

embryonic diploid cells (HEL-12V). Human sera did not react with normal gibbon spleen monolayer cells (GSpl) but showed positive reactions with non-producing human embryonic diploid cells CIH-1(V-) which become virus-producing cells CIH-32(V^+) after repeated passages, whereas gibbon sera reacted with GSpl but not with CIH-1(V-). Human sera strongly reacted with viral envelope antigen (VEA) of HL-23V common to VEA of GALV, but gibbon sera reacted weakly with it. Human sera cross-reacted with nonproducing human acute lymphocytic leukemia blood cell lines, but gibbon sera did not.

Some of the human sera reacted weakly with VEA of feline leukemia virus (FeLV) in the direct IEM, but their reactivity of VEA of GALV was not removed by FeLV-associated surface antigens. Some human sera cross-reacted with VEA of GALV and Rauscher-MuLV.

Seventy-nine percent of 76 sera from healthy persons were positive for VEA of GALV with various antibody activity. A peak of antibody level and incidence of positive sera was observed at ages 20 to 50 years, which corresponds inversely to a leukemia incidence with two peaks at ages 1 to 14 years and again over 50 years.

2. Peripheral blood platelets of mice, humans, and quail contained growth-stimulating factors (TSF) and growth-inhibiting factors (TIF) of type C virus-induced primary tumors in mice and/or chicken. The platelet's property to stimulate the tumor growth was not species-specific. TSF could be readily released from the platelets by a homogenization procedure, and TIF was frequently released by storage at 4°C for at least 7 days. After these treatments, the remaining platelets could still enhance the tumor growth. Both TSF and TIF could be easily filtered through 0.45 mμ Swinney filters.

3. A variety of transplantation mouse tumor lines were shown to contain murine type C viruses and virus-associated antigens. The type of virus isolated and antigens detected could not invariably be correlated with the original method of tumor induction, but testing of the majority of tumor lines for infectious virus at various levels of in vivo or in vitro passage yielded isolates that were consistent in tissue culture host range for each tumor. In contrast, during the course of in vivo transplantation, some of the lines underwent considerable change in the pattern of virus-associated cell-surface antigens. When the transplanted tumor lines were placed into culture, they showed some alteration in the detectable antigens. Upon retransplantation and passage of the cultured cells into mice, the surface antigens gradually returned to the original in vivo patterns and occasionally acquired additional type C virus-associated antigens not detected in the original tumor line. To test for association of antigens with infectious virus, appropriate tissue culture cell lines were infected with the viruses isolated from the tumors. In these infected indicator cells, some new virus-associated cell-surface and virion envelope antigens were detected, but the complete array of antigens found in the original tumor lines was not acquired.

4. (C57BL/6 x C3Hf/He)F1 anti-AKR spontaneous leukemia sera were preabsorbed with nonleukemic AKR lymphoid cells to remove antibodies against virus-associated surface antigens and alloantigens. In absorption tests the antisera showed no cross-reaction by immunofluorescence microscopy with thymocyte, fetal, male-specific H-Y, E, and X.1 antigens; with known Gross murine leukemia virus-associated antigens; or with cell surface antigens on Friend, Moloney, and Rauscher MuLV-induced tumors. It was thus shown that the antigens detected were leukemia-specific, were acquired by malignant transformation, and consisted to two types: (a) a common antigen in all AKR leukemias, and (b) individual antigens found in several leukemias which showed distinct patterns of partial cross-reactivity with the other leukemias. These AKR leukemia specific antigens have been observed by chemical approach to be gp75 on cell surface. This gp75 was not present on AKR virus-infected nonmalignant cells, and showed cross-reaction with p30 and p15(E). The gp75 may carry other unknown antigenic determinants which might be AKR leukemia specific antigenic determinants.

5. When 2- and 5-month-old AKR mice were inoculated i.m. with the coccus preparation twice weekly throughout their lifespans, spontaneous leukemias occurred later and at a low incidence than in control groups. By virus neutralization and cytotoxic tests and by IEM, humoral antibodies against virus and cell-surface antigens of transplanted AKR spontaneous leukemia were not detectable in sera of nonleukemic mice of any group. Whereas sera from mice treated with the coccus were the only ones positive for interferon, viremia was clearly demonstrated in control groups by reverse transcriptase assay of the plasma.

The inoculation of the coccus preparation increased the cell-mediated immunity in 6-month-old AKR mice.

Significance to Biomedical Research and the Program of the Institute:

1. The presence of natural antibodies in sera from humans and gibbon apes against type C viruses from primates suggests that the foot-prints of human type C virus may exist. The specificity of natural antibodies is quite broad, indicating the possibility of viral etiology, immunoprevention, and immunotherapy of human tumors. These results meet one of the final major goals of viral etiology research in humans.

2. The purification of stimulating and inhibiting factors of type C virus-induced primary tumor growth will be very useful for interpreting the mechanisms of tumor growth. Furthermore both the tumor growth-stimulating factors (TSF) and the tumor growth-inhibiting factor (TIF) would be very useful in clinical treatment; the inoculation of either antibody against TSF or of TIF itself may inhibit hyperplasia.

3. The alteration in the expression of several different type C viruses in long transplanted cell lines or long cultured cell lines was clarified. These results draw attention of investigators to examine carefully the expression of type C virus-associated surface antigens for avoiding the misinterpretation of results obtained by using long-term maintained tumor lines.

4. Classical immunological approach provides a good guidance for molecular immunological approach which sometimes changes antigenic determinants by chemical purification or identification. The presence of malignant cell-specific antigens may open a way to establishing the method for diagnosis, etiology, and treatment of malignant diseases.

5. The treatment with the coccus preparation is somewhat promising in some tumors, especially type C virus-induced tumors, as the nonspecific immunotherapy of tumors, particularly when this is combined with chemotherapy.

Proposed Course:

1. To confirm the presence of putative human type C virus by analyzing the specificity of naturally occurring antibodies in human sera.

2. To isolate and to characterize chemically the tumor growth-stimulating factor and the tumor-inhibiting factor from peripheral blood platelets of easily obtainable sources such as cow, horse, etc. for prevention or treatment of malignant diseases.

3. To provide some approach for better understanding of results obtained by using long-term transplanted tumor line or long-term cultured cell lines.

4. To bridge tightly between classical and molecular immunologies.

5. To enhance host immune response to type C virus-induced tumors by the combined use of nonspecific immunotherapy and conventional chemotherapy.

Publications:

Aoki, T., Liu, M., Walling, M. J., Bushar, G. S., Brandchaft, P. B. and Kawakami, T. G.: Specificity of naturally occurring antibody in normal gibbon serum. Science 191: 1180-1183, 1976.

Aoki, T., Kvedar, J. P., Hollis, V. W., Jr. and Bushar, G. S.: Streptococcus pyogenes preparation OK-432: Immunoprophylactic and immunotherapeutic effects on the incidence of spontaneous leukemia in AKR mice. J. Natl. Cancer Inst. 56: 687-690, 1976.

Okazaki, E., Aoki, T. and deKozlowski, S. J.: AKR leukemia-specific surface antigens acquired by malignant transformation: their common and indi idual specificities. Int. J. Cancer 17: 640-676, 1976.

Brandchaft, P. B., Aoki, T. and Silverman, T. N.: Immunogenic and immunosuppressive components in soluble preparation from Moloney murine leukemia virus-induced tumor cells. Int. J. Cancer 17: 678-685, 1976.

Aoki, T., Walling M. J., Bushar, G. S., Liu, M. and Hsu, K. C.: Natural antibodies in sera from healthy humans to antigens on surfaces of type C RNA viruses and cells from primates. Proc. Natl. Acad. Sci. USA 73: 2491-2495, 1976.

Aoki, T., Herberman, R. B., Hartley, J. W., Liu, M., Walling, M. J. and Nunn, M.: Surface antigens on transplantable tumor cell lines producing mouse type C viruses. J. Natl. Cancer Inst. (In Press).

Aoki, T.: Serological approach to the detection of putative human type C RNA virus. In Gallo, R. (Ed): Cancer Research: Cell Biology, Molecular Biology, and Tumor Virology. Cleveland, Ohio. CRC Press, Inc. (In Press).

Aoki, T., Liu, M., Walling, M. J., Bushar, G. S. and Hsu, K. C.: Human and gibbon natural antibodies to envelope antigens of type C viruses from primates. Excerpta Medica (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04866-02-LVC

PERIOD COVERED
July 1, 1976 to April 8, 1977

TITLE OF PROJECT (80 characters or less)
Immunological Effects of Cryosurgery and Adoptive Transfer of Immune Lymphocytes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H. Kimura	Visiting Fellow	LVC NCI
OTHER:	T. Aoki	Head, Immunology Section	LVC NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Immunology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to study the immunological effects of Cryosurgery on Moloney murine sarcoma virus (M-MuSV)-induced tumors in mice. The effect of cryosurgery was compared with that of conventional surgery in terms of the following aspects: tumor growth, cumulative mortality, proliferative response of immune lymphocytes, cell-mediated cytotoxicity and humoral antibody formation against tumor cells. Cryosurgery enhanced the immune response of host animals and reduced the tumor incidence and the death of sarcoma invasion.

The effect of adaptive transfer of lymphocytes to M-MuSV-induced tumor-bearing mice was studied in terms of the tumor growth. Spleen cells from mice 12 days after virus-inoculation enhanced the tumor growth if they were transferred after tumor development, whereas the same lymphocytes inhibited tumor development if they were transferred 7 days before virus inoculation. Furthermore, these enhancing and inhibiting effects were antigenically specific and mediated by thymus-dependent cell population. The kinetics of these two paradoxical effects were investigated.

1792

Project Description

Objectives:

1. To study the immunological effects of cryosurgery on M-MuSV-induced primary sarcomas.

2. To study the relation of immunological effects detected by *in vitro* techniques with the *in vivo* results.

3. To study the effect of immune lymphocytes on growth of tumors induced by M-MuSV.

Methods Employed:

1. Cryosurgery, a method originally developed in clinical field to treat prostate cancer, was used to freeze and to destroy sarcomas induced by M-MuSV in BALB/c mice.

2. Observation of tumor growth and cumulative mortality. Cell-mediated immune response was determined by lymphocyte mediated cytotoxicity assay (^{125}IUdR-release), and ^{3}H-thymidine (^{3}HtdR)-incorporation tests by lymphocytes. Humoral antibody formation was measured by immunofluorescence microscopy.

3. Adaptive transfer of lymphocytes: intravenous injection and intraperitoneal injection of normal and immune lymphocytes. Observation of the tumor growth.

Major Findings:

1. and 2. Cellular immune response detected by ^{3}H-TdR-incorporation in lymphocytes was enhanced significantly by cryosurgery of the primary sarcoma. Peak activity was observed 3 days after cryosurgery, whereas marked inhibition of ^{3}H-TdR-incorporation was observed in the group of mice treated with conventional surgery. In the lymphocyte-mediated cytotoxicity detected by ^{125}IUdR-releasing assay, cryosurgery, also demonstrated significant enhancement of specific immune response to virus-associated antigens on the target cells compared to the response by surgical operation or no treatment. *In vivo* effects were not so distinctive as *in vitro* effects, but cryosurgery of primary tumor indicated better trends in the tumor growth and cumulative mortality.

3. One x 10^{6} spleen cells from donor mice 12 days after virus inoculation inhibited the tumor growth significantly if they were inoculated intraperitoneally into recipient mice 7 days before virus inoculation, whereas 5 x 10^{7} lymphocytes were required to inhibit the tumor growth in intravenous inoculation at the same time period. Significant enhancement was observed with the same lymphocytes (2 x 10^{8} - 5 x 10^{7}) if they were inoculated to tumor bearing (8 days after virus inoculation) mice intraperitoneally but

not intravenously. Nylon-wool-column purification of these lymphocytes revealed that both the enhancing and inhibiting effects were mediated by thymus dependent lymphocytes. Furthermore, these effects were immunologically specific, because these lymphocytes neither inhibited nor enhanced the growth of Methylcholanthrene-induced sarcoma MCA-15B. The kinetics of these enhancing and inhibiting effects are now under investigation.

Significance to Biomedical Research and the Program of the Institute:

1. and 2. Cancer patients cannot usually mount an effective immune response to their own tumors. By stimulating the immune system of the host with tumor specific antigens, we may be able to enhance a specific immune response against tumor cells. Our findings that the cryosurgery enhanced specific immune response against the M-MuSV-induced primary tumors may provide us with the better methods to provoke an effective response in the host. The utilization of the primary tumors instead of the transplanted ones may also provide us with the better understanding of the clinical cases.

3. In tumor immunology, it has been reported by many investigators that tumors possess specific antigens which are recognized and induce immune response in hosts detected in the variety of in vitro systems. Despite these immune responses observed in vitro, most tumors grow progressively until death of the host animals. Various reasons have been proposed to explain the descrepancies between these in vitro and in vivo results; (a) specific serum blocking factors in tumor-bearing mice, (b) suppressor cell activity against specific immune lymphocytes, and (c) cell-mediated enhancement of tumor growth.

To analyze further the possibilities mentioned above, it will be necessary to develop systems which will enable us to investigate the direct in vivo effect of immune lymphocytes on tumor growth with as little modifications by artifacts as possible. Our system, which includes adaptive transfer of lymphocytes to M-MuSV-induced tumor-bearing mice and the observation of resulting modulation of tumor growth pattern, is quite simple and less artificial than other systems.

Proposed Course:

1 - 3. These projects have been accomplished as of April 8, 1977.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04867-02-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Receptors for Nerve Growth Factor (NGF) on Human Melanomas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R. N. Fabricant	Research Associate	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	J. E. De Larco	Senior Staff Fellow	LVC, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Human melanomas in culture possess large numbers of receptors for NGF. This finding is consistent with the embryologic origin of these cells as they are derivatives of the neural crest. The significance of this observation in regard to the pathophysiology of pigment cell disease is actively studied.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Work is directed at obtaining a means of inhibiting the growth of human melanomas by studying their specific, surface receptors for nerve growth factor.

Methods Employed:

Methods used include the use of iodinated preparations of NGF in *in vitro* binding assays, as well as radioimmunoassays. Ultracentrifugation, gel filtration and electrophoresis are implemented.

Major Findings:

Like sympathetic ganglia, embryonic sensory ganglia, and mouse neuroblastomas, human melanomas possess receptors for NGF. As determined by a survival assay, these receptors are biologically active.

Significance to Biomedical Research and the Program of the Institute:

The finding of NGF receptors of human melanomas provides a specific surface marker on this highly metastatic and aggressive type of neoplasm. This marker may provide a mechanism for specifically attacking this tumor by immunologic means in the clinical setting. The possibilities of using the finding for diagnostic purposes and tumor response during therapy remain to be thoroughly explored.

Proposed Course:

Correlation of NGF binding to human melanomas with the clinical course is now being actively pursued. The use of the NGF receptor as a possible means of inhibiting tumor growth is also being studied.

Publications:

Fabricant, R.N., De Larco, J.E. and Todaro, G.J.: Nerve growth factor receptors on human melanoma cells in culture. *Proc. Nat. Acad. Sci. USA* 74: 565-569, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04868-02-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Endogenous Type C Viruses in Spontaneous and Chemically Mediated Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	U. R. Rapp	Visiting Scientist	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	J. E. De Larco	Senior Staff Fellow	LVC, NCI
	R. Callahan	Staff Fellow	LVC, NCI
	T. H. Marshall	Expert	LVC, NCI
	A. Sen	Visiting Fellow	LVC, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.0	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The endogenous ecotropic virus of the low leukemic strains of mice C3H and BALB/c is poorly infectious and of low pathogenicity. Cells infected with these viruses produce gs antigen and express GCSA (Gross Cell Surface Antigen) on their membranes but not GIX and production of virus particles is exceedingly low. The level of gp70 produced in these cells is 10- to 100-fold below the level found in productively infected cells whereas p30 antigens are present in comparable quantities. Many of the cells that are infected with replication deficient virus segregate gs antigen negative cells in subsequent cell divisions.

These poorly infectious viruses can be converted to a replication competent pathogenic form when grown in certain transformed mouse cells. This conversion involves several mutations and possibly recombinational events. The replication competent virus is able to activate xenotropic virus in infected cells and is pathogenic in NFS mice.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

Type C viruses from mice, cats and primates will be isolated and characterized with respect to their biochemical properties as well as their biological activities in vitro and, where possible, in vivo. Mechanisms regulating the expression of these viruses will be studied in in vitro activation experiments. The genetics of virulence of these viruses and their interaction with the cellular genome will be analyzed.

Methods Employed:

Cell lines from inbred strains of mice, cats and primates will be used in in vitro transformation experiments with chemical carcinogens and selected growth conditions. Expression of type C viral functions during the action of the oncogenic agent as well as in clonal cell lines isolated from individual foci of stably transformed cells will be analyzed by immunofluorescence with viable and fixed cells, radioimmunoassays for viral structural proteins, DNA:RNA hybridization. Biological properties of isolated viruses from nontransformed and transformed cells will be studied in in vivo tumorigenicity experiments. Their effect on growth properties of established cell lines susceptible to infection as well as their effect on transformation frequencies of established cell lines will be determined in quantitative in vitro carcinogenesis and cocarcinogenesis experiments. Virus isolates of low virulence will be altered by mutation and recombination and the genetic changes leading to more pathogenic forms will be analyzed by hybridization, oligonucleotide fingerprinting, and serological characterization of viral proteins.

Major Findings:

Whereas high leukemic mouse strains were found to carry a highly infectious endogenous ecotropic virus, the ecotropic virus from several low leukemic strains was only poorly infectious. These replication deficient viruses have a very limited host range which excludes rat cells but show a high specific infectivity for mouse cells in which they are expressed as GCSA and cytoplasmic p30 antigen. However, GIX cell surface antigen (other forms of gp70 and gp70 precursors were only barely detectable in infected cells and particle production) was exceedingly low. The pathogenicity of these viruses is low as determined by injection into newborn mice of susceptible strains. The replication deficient viruses were converted into a virulent form by passage in certain transformed cells. This conversion was accelerated by mutagens and requires several cycles of infection. Xenotropic virus was activated in the process of conversion. The converted virus is replication competent, has a widened host range which now includes rat cells, is positive in the XC test, and induces GCSA as well as GIX in infected cells. This virus is efficient in activating xenotropic virus in infected cells in vitro and in vivo and is pathogenic in NFS mice. The genetic changes which

are associated with these alterations in biologic activity are not detectable by hybridization but are apparent on oligonucleotide fingerprints of the viral RNA genome.

The replication competent ecotropic viruses have been found to be active in in vitro cocarcinogenesis experiments.

Significance to Biomedical Research and the Program of the Institute:

These studies will facilitate the isolation of new type C viruses and provide knowledge concerning the regulation of their expression and their possible involvement in transformation.

Proposed Course:

The genetic changes that are required for the virulence of endogenous mouse type C viruses will be further analyzed by serological and biochemical methods. Virulent viruses from rodents and primates will be used as activating viruses in superinfection activation experiments with rodent and primates in vitro and, where possible, in vivo. The ability of these viruses to act as cocarcinogens in in vitro transformation will be studied using a quantitative chemical carcinogenesis assay.

Publications:

Rapp, U.R. and Nowinski, R.C.: Endogenous ecotropic mouse type C viruses deficient in replication and production of XC plaques. J. Virol. 18: 411-417, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04869-02-LVC

PERIOD COVERED
July 1, 1976 to January 31, 1977

TITLE OF PROJECT (80 characters or less)

Function of the Low Molecular Weight Proteins of RNA Tumor Viruses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Sen	Visiting Fellow	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	C. J. Sherr	Medical Officer	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

All the mammalian and avian type C viruses contain a major structural phosphoprotein. The purified phosphoprotein from each virus specifically binds to the homologous viral RNA genome. Direct and competition binding assays have been developed to study the specificity of such protein-RNA interaction in the different classes of type C viruses. The phosphoprotein from each virus can be subfractionated on the basis of the extent of phosphorylation. Such modification has been shown to modulate the extent but not the specificity of its binding to the viral RNA genome. A subpopulation of the viral structural phosphoprotein, characterized by a low level of phosphorylation, can be specifically recovered from the intact, crosslinked 70S genome of UV-irradiated type C viruses. The close association with the viral genome, the ability of specific interaction with homologous viral RNA and the covalent modification (phosphorylation) suggest a possible regulatory role of the type C viral phosphoprotein in host-virus interaction.

PHS-6040
(Rev. 10-76)

Project Description

An understanding of the functional characteristics of the type C viral proteins would be of major importance in studying the host cell-virus interactions. We have characterized the major structural phosphoprotein of the avian and mammalian type C viruses as the specific homologous viral RNA binding protein.

Several independent methods of purification of this protein have been developed. One approach is to subject detergent-disrupted virion proteins to Sepharose gel filtration in the presence of 6 M guanidine hydrochloride. A second method is polyethylene glycol extraction of viral suspensions disrupted with a nonionic detergent in the presence of high concentrations of salt and subsequently fractionating the protein supernatants by gel filtration. It was observed that proteins purified by the latter method retained a higher ability to recognize homologous viral RNA in binding assays. Phosphorylation of this specific RNA binding protein was seen to influence the extent but not the specificity of the interaction in vitro. A subpopulation of this protein characterized by a low level of phosphorylation could be specifically recovered in close association with the 70S viral genome stabilized by UV-irradiation of the intact virion.

The specificity of binding of this RNA binding protein, the close association of a specific fraction of a packaged phosphoprotein molecule with the viral genome in situ and the effect of covalent modification (phosphorylation) of the protein on the extent of binding all suggest a key role of this protein in the life cycle of type C viruses.

In the future, we will attempt to further characterize the binding interaction and to study the effect of this binding on the replication and expression of the viral genes.

Publications:

Sen, A. and Todaro, G.J.: Specificity of in vitro binding of primate type C viral RNA and the homologous viral p12 core protein. Science 193: 326-328, 1976.

Sen, A. and Todaro, G.J.: The genome-associated, specific RNA binding proteins of avian and mammalian type C viruses. Cell 10: 91-99, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04870-01-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Genetics of Mammalian Virus Expression

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. H. Marshall	Expert	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	R. E. Benveniste	Microbiologist	LVC, NCI
	R. Callahan	Staff Fellow	LVC, NCI
	J. E. De Larco	Senior Staff Fellow	LVC, NCI
	C. J. Sherr	Medical Officer	LVC, NCI
	U. R. Rapp	Visiting Scientist	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The concept of permanently integrated type C viral genes in somatic cells is well established. Their expression may be under tight genetic control. Relaxation of this control appears related to onset of neoplasia and cell transformation. Thus, it is important to understand the relations among various virogenes and genes which code for viral related functions. The principal method of this investigation is that of interspecies somatic cell hybridization. Hybrid cells are constructed from a variety of primary and established cell lines such that chromosomes of one parent are preferentially or exclusively lost. This permits linkage studies of viral gene elements and growth factor binding and response coded by the chromosomes being segregated. The results show that it is possible to construct the following classes of hybrid cells suitable for mapping integration sites of infectious and endogenous viruses: (1) rodent X primate (including human); (2) Mus cervicolor and Mus caroli X Mus musculus and Chinese hamster; (3) Mus musculus X Chinese hamster. All of these hybrid cells are of course useful for formal genetic studies.

Project No. Z01-CP-04870-01-LVC

Project Description

Objectives:

To study the organization of and interactions among virogenes, viral related functions, and growth factor binding and responses in normal and tumor cells.

Methods Employed:

The major approach is that of somatic cell hybridization. A variety of interspecies somatic cell fusions are performed using Sendai virus and polyethylene glycol. The hybrid clones are picked and expanded in selective media. Soluble extracts are analyzed by starch gel electrophoresis and histochemical staining to visualize parental and hybrid isozymes. Thus the extent and nature of isozyme (chromosome) segregation is characterized. In collaboration with other investigators virogenes are assayed by molecular hybridization and antigens by radioimmunoassay.

Major Findings:

It is possible to routinely produce hybrid cells which exclusively segregate chromosomes of one parental species predetermined so as to facilitate studies of virogenes and viral gene products. For example, human cells productively infected with four type C viruses have been fused to rodent cells. Chimpanzee X rodent isozymes have been produced. Hybrids among various Mus species and Chinese hamster segregating caroli and cervicolor chromosomes should allow mapping of various endogenous virogenes. Most of the isozymes which can be used to characterize segregation in Mus musculus X Chinese hamster hybrid cells can be distinguished in hybrid cells of Southeast Asian species of Mus. Numerous hybrid clones of low leukemic inbred strains of laboratory mice fused to Chinese hamster have been produced. Cell hybridization is based on selective gene markers, i.e., mutants selected for drug resistance. Twelve 6-thioguanine resistant caroli kidney clones have been produced. Two independently derived caroli cell lines have been found resistant to toxic levels of diaminopurine, an antitumor agent.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04883-05-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Cancer Immunodiagnosis and Immunotherapy. Cellular Mechanisms of Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	C.W. Boone, MD.	Medical Officer	LVC	NCI
OTHER:	N. Takeichi, M.D., Ph.D.	Expert Consultant	LVC	NCI
	M. Paranjpe, Ph.D.	Senior Staff Fellow	LVC	NCI

COOPERATING UNITS (if any)
R. Gillette, Director of Basic Research, Comprehensive Cancer Center, Honolulu Hawaii and Edmund Klein, Chief, Dermatology Service, Roswell Park Memorial Institute, Buffalo, New York

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. Improved human cancer skin test. Membranes from cultured human melanoma cells infected with vesicular stomatis virus and UV inactivated gave markedly more sensitive skin test reactions in melanoma patients. 2. Methycholanthrene induced squamous cell carcinomas showed cross reacting tumor rejection antigens. This represents a new finding compared to previous reports that methylcholanthrene induced sarcomas do not have cross reacting antigens. 3. Mouse model for cancer vaccine preparations from tumor cells. Cell membrane preparations from influenza virus infected tumor cells induced strong tumor protection; membranes from non-infected tumor cells induced no tumor protection at all. The dose, route and timing of "virus-augmented" tumor cells membrane antigens to achieve optimum tumor immunity was determined. 4. Spontaneous neoplastic transformation in vitro is a modified form of foreign body tumorigenesis. Cultured cells such as Balb/3T3, $C_3H/10\frac{1}{2}$ or recently explanted adult subcutaneous connective tissue were not tumorigenic when inoculated subcutaneously in fluid suspension, but are definitely tumorigenic if inoculated attached to 1x5x10 mm plastic plates.

Project Description

Objectives:

1. To develop improved human cancer skin test antigens.
2. To develop improved immunodiagnostic and immunotherapeutic techniques in animal model systems of potential application to humans.
3. To elucidate the mechanisms of carcinogenesis at the cellular level.
4. To identify attributes of tumor cells specifically responsible for malignant behavior.

Methods Employed:

Tumor-graft rejections assay, delayed hypersensitivity assays against tumor antigens, *in vitro* assays for humoral and cell-mediated immunity against tumors. Tissue culture and cinematographic procedures associated with tumorigenicity *in vivo*. Protein biochemistry procedures.

Major Findings:

1. A markedly improved human cancer skin test antigen was developed. Cell membranes from cultured human melanoma cells infected with vesicular stomatitis virus and UV inactivated gave remarkably stronger skin test reactions compared to membranes from uninfected melanoma cells in 10 human melanoma patients who had previously had their tumors surgically removed. The reactivity was specific as shown by the fact that patients with other cancers did not show skin test reactivity.

2. In cancer vaccine studies in the mouse model systems, the dose, route, and timing of immunization with "virus-augmented" tumor cell membrane antigens (i.e., membranes isolated from cells infected with a surface budding virus such as influenza or vesicular stomatitis virus), was determined. Tumor cell membranes not "virus-augmented" induced no tumor immunity.

3. Methylcholanthrene induced squamous cell carcinomas in mice showed cross reacting transplantation rejection antigens. Previous reports had shown that MCA induced sarcomas did not cross react antigenically. Ours is the first report that MCA induced *carcinomas*, rather then *sarcomas*, do show cross reacting antigens.

4. Spontaneous neoplastic transformation *in vitro* is a modified form of foreign body tumorigenesis. This year's results have lead us to conclude that the concept of "normal" mouse cells in culture may be an invalid one. Instead, they should be viewed as being in the continual process of progression toward spontaneous neoplasia induced by the same causal factor that initiates foreign body solid surface tumorigenesis *in vivo*: the presentation to the cells of an extensive flat surface on which to attach and grow in the absence of an adequate blood supply. When explants

of adult mouse subcutaneous connective tissue were tested for tumorigenicity every two weeks by subcutaneous inoculation of cells either attached to 1x5x10 mm plastic plates or suspended in saline, the plate-attached cells became tumorigenic sooner and in lower dosage. This effect of plastic plate attachment was not due to greater concentration of the cell inocula *in vivo* because the minimum tumorigenic dose of a separate line of tumor cells was not decreased by plastic plate attachment even at doses as low as 50 cells.

Significance to Biomedical Research and Program of the Institute:

1. Development of human cancer skin test antigens of increased sensitivity. The initially high expectations for the potential effectiveness of cancer immunotherapy in humans a decade or more ago is now being tempered by the rapidly growing number of clinical immunotherapy trials in which the results are meager or negative. A major reason for this outcome is that the presence, and in particular the strength, of tumor antigens in the different types and grades of human cancer has not been sufficiently established. It is clear that without the existence of tumor antigens, both non-specific immunotherapy, such as with BCG, and specific immunotherapy with an antigen vaccine would be impossible. It is therefore important that the major types of human cancer be systematically screened for the presence and strength of tumor antigens. Skin testing with cancer antigens appears to offer the best method for this type of screening. The development of a technique for markedly increasing the sensitivity of cancer skin test antigens enhances the chances of success for such screening.

2. Antigenic cross reactivity of methylcholanthrene induced skin cancer antigens. Without antigenic cross reactivity there is little hope for immunotherapy. The finding that chemically induced skin cancer antigens cross react supports the possibility that shared tumor antigens will also be found in squamous cell carcinomas in humans (lung, cervix, esophagus, oropharynx, lower urogenital tract).

3. Spontaneous neoplastic transformation in vitro is a modified form of foreign body tumorigenesis. The demonstration that the process of tissue culture per se is progressively sarcomagenic to mouse connective tissue cells because of exposure to the solid flat surface of the culture vessel should induce a reorientation in the thinking of many scientists who are currently reporting on differences between "normal" and "transformed" cells in vitro. Concepts such as "tumor progression", "preneoplastic maturation", and the requirement for the acquisition of multiple new attributes before malignancy can be fully expressed will now have to be more fully considered by these scientists.

Proposed Course:

1. Human cancer skin test antigens. The studies in human melanoma patients will be extended to confirm initial positive findings. Patients with carcinoma of the lung and breast will next be tested with "virus-augmented" skin test antigens.

2. Cross reactivity of antigens of methylcholanthrene induced squamous cell carcinoma. Because of the milestone significance of this finding, and with the encouragement of a number of prominent tumor immunologists, this is being repeated in spite of the laboriousness involved.

3. Spontaneous neoplastic transformation in vitro. Careful studies delineating the specific cellular traits that are responsible for malignant cell behavior will continue. Mechanisms of invasiveness will also be pursued.

Publications:

1. Takeichi, N., Economou, G.C. and Boone, C.W.: Accelerated regeneration of trypsin-treated surface antigens of SV40 transformed BALB/3T3 cells induced by X-irradiation. Cancer Res., 36: 1258-1262, 1976.

2. Boone, C.W., Takeichi, N., Paranjpe, M.S. and Gilden, R.: Vasoformative sarcomas arising from BALB/3T3 cells attached to solid substrates. Cancer Res., 36: 94-101, 1976.

3. Takeichi, N., Economou, G.C. and Boone, C.W.: Local adoptive transfer of anti-tumor cellular immunity to xenogeneic animals studied with a radioisotopic footpad assay. J. Natl. Cancer Instit., 56: 131-134, 1976.

4. Yoshida, M.C., Sasaki, M., Takeichi, N. and Boone, C.W.: Karyotypes of vasoformative sarcomas arising from BALB/3T3 cells attached to polycarbonate plates. Cancer Res., 36: 2235-2240, 1976.

5. Paranjpe, M.S., Boone, C.W. and Takeichi, N.: Specific paralysis of the anti-tumor cellular immune response produced by growing tumors studied with a radioisotopic footpad assay. Annals of the New York Academy of Sciences, 276: 254-259, 1976.

6. Takeichi, N., Boone, C.W. and Klein, E.: Local adoptive transfer to mice of human delayed hypersensitivity reactions by the radioisotope footpad assay. Clin. Exp. Immunol., 26: 310-314, 1976.

7. Takeichi, N., Kodama, T., Kobayashi, H. and Boone, C.W.: Adoptive immunotherapy of Gross virus producing lymphomas and methylcholanthrene-induced fibrosarcomas in tolerant rats. Cancer Res., 36: 4039-4043, 1976.

8. Economou, G.C., Takeichi, N. and Boone, C.W.: Common tumor rejection antigens in methylcholanthrene-induced squamous cell carcinomas of mice detected by tumor protection and a radioisotopic footpad assay. Cancer Res., 37: 37-41, 1976.

9. Takeichi, N. and Boone, C.W.: Spontaneous rosette formation of rat thymus cells with guinea pig erythrocytes. Cellular Immunol., 27: 52-59, 1976.

10. Gillette, R.W. and Boone, C.W.: Augmented immunogenicity of tumor cell membranes produced by surface budding viruses. Parameters of optimal immunization. Int. J. Cancer, 18: 212-222, 1976.

11. O'Brien, S.J., Boone, C.W., Simonson, J.M. and Austin, F.C.: The paired label assay for cell surface antigens. Tissue Culture Association Manual, 2: 423-427, 1976.

12. Boone, C.W. and Jacobs, J.B.: Sarcomas routinely produced from putatively non-tumorigenic Balb/3T3 and C3H/10T½ cells by subcutaneous inoculation attached to plastic platelets. J. Supermolecular Structure (In Press).

13. O'Brien S.J., Simonson, J.M. and Boone, C.W.: Expression of viral structural protein on murine cell surfaces in association with the production of murine leukemia virus. J. Gen. Virol. (In Press).

14. Williams, R.E., Linsley, P.S., Fox, F., Rittenhouse, H.G. and Boone, C.W.: Lack of constant correlation between normal phenotypic properties and tumorigenicity in variant cell lines. Cell (In Press).

15. O'Brien, S.J. and Boone, C.W.: Expression of feline leukemia virus antigens on cat lymphoma cells: Kinetics of biosynthesis. J. Gen. Virol. (In Press).

16. Boone, C.W.: Comment on comparing the membrane transport properties of "Normal" & "Transformed" Balb/3T3 cells. J. of Cell. Phys. (In Press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04892-06-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
In Vitro Studies on Cellular Mechanisms of Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Meera S. Paranjpe, Ph.D.	Senior Staff Fellow	LVC	NCI
OTHER: Charles W. Boone	Medical Officer	LVC	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The transition of the non-tumorigenic $C_3H10T_{½}$ cells from a state of controlled growth to the neoplastic state was achieved under conditions of in vivo selection pressure by subcutaneous implantation of these cells attached to 1x5x10 mm plastic plates. Cell lines from four different $C_3H10T_{½}$ tumors so formed explanted back in vitro showed coordinate loss of density inhibition of proliferation, anchorage dependence, and high serum growth requirement. This was further evidence that these changes were an integral part of the process of neoplastic progression in vivo. The degree of loss of density, anchorage, and serum regulation by the tumor cell lines in the first five passages tended to correlate the degree of their growth aggressiveness in vivo. Plasminogen activator activity, though present, did not follow this general correlation with degree of tumor growth aggressiveness. A useful tumor growth aggressiveness index, incorporating both parameters of tumor incidence and latent period, was formulated. Non-tumorigenic cells were observed to be blocked in portion of cell-cycle between metaphase & early G_1, when grown on a non-adhesive substrate like Teflon.

Project Description

Objective:

Correlation of *in vitro* phenotypic alterations of cultured cells with their tumorigenic potential. This includes investigations of cellular characters responsible for invasive behavior of malignant cell lines.

Methods Employed:

Tissue culture techniques for *in vitro* studies, chick allantoic membrane assay and tumor rejection assay for *in vivo* studies. Immunofluoresence, various biochemical assays, time-lapse microcinematography as required.

Major Findings:

The transition of the non-tumorigenic $C_3H10T½$ cells from a state of controlled growth to the neoplastic state was achieved under conditions of *in vivo* selection pressure by subcutaneous implantation of these cells attached to 1x5x10 mm plastic plates. Cell lines from four different $C_3H10T½$ tumors so formed explanted back *in vitro* showed coordinate loss of density inhibition of proliferation, anchorage dependence, and high serum growth requirement. This was further evidence that these changes were an integral part of the process of neoplastic progression *in vivo*. The degree of loss of density, anchorage, and serum regulation by the tumor cell lines intended to correlate with the degree of their growth aggressiveness *in vivo*. Plasminogen activator activity, though present, did not follow this general correlation with degree of tumor growth aggressiveness. A useful tumor growth aggressiveness index, incorporating both parameters of tumor incidence and latent period, was formulated. Cells from the $P10C_4$ cell line which showed the most aggressive tumor growth when implanted on the chick allantoic membrane to observe its invasive properties, formed well defined macroscopic tumors within 4 days.

Normal non-tumorigenic cells grown on methylcellulose and Teflon were observed to be blocked in the portion of the cell cyle between metaphase and early G_1. The cells do not die on Teflon: their viability remained unchanged up to 4 to 6 days with respect to plating efficiency, vital staining, and resumption of normal growth when transferred to suitable plastic or glass substrates. Twenty to 24 hours after transfer of cells from Teflon to plastic substrates, cells began to traverse the S phase of the cell cyle.

Proposed Course:

1. Studies on invasiveness. The early passages of all cell lines established from explanted tumors originating from $C_3H10T½$ cells will be studied for their invasive properties on the chick allantoic membrane. If invasive, these lines will also be studied for breakdown of collagen to see if degree of collagen breakdown relates with degree of tumorigenicity *in vivo*

2. Establishment of a non-tumorigenic epithelial cell line in continuous culture. It is aimed to establish a continuously growing cell line of murine non-tumorigenic epithelial cells in culture for purposes of studying transformation in vitro. A number of criteria have been established to characterize malignant fibroblast cell lines. It is proposed to validate these same criteria with respect to non-tumorigenic epithelial cell lines in culture, and also to see to what extent they are applicable to benign and invasive tumors.

3. Studies on hybrids of malignant and normal cells. In order to explore the possibility of complementation between malignant tumors and normal non-tumorigenic cells, hybrids of these two cell types will be studied for expression of other in vitro correlates of malignancy besides tumorigenicity.

4. Non-tumorigenic fibroblasts were blocked between the metaphase and G_1 phase of the cell cycle when suspended on a methylcellulose Teflon substrate. These cells were grown in fetal calf serum which contains a number of serum inhibitors of plasminogen. Since anchorage independent growth of transformed cells is associated with production of a plasminogen activator, it is proposed to observe if the cell cycle block can be overcome by culturing them in the presence of sera which permit high levels of plasmin formation and activity. The effect of other proteases will also be studied.

Significance of Proposed Research:

Studies on the cellular mechanisms resulting in neoplastic transformation are essential to understanding the progression of normal precancerous cells to cells capable of forming benign and invasive tumors.

Publications:

1. Paranjpe, M.S., Boone, C.W., and Takeichi, N.: Specific paralysis of anti-tumor cellular immune response produced by growing tumors studied with a radioisotopic footpad assay. Annals of the New York Academy of Sciences. 276: 254-259, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04896-05-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analysis of Gene Controlled Events in Neoplastic Transformation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S.J. O'Brien	Staff Fellow	LVC NCI
OTHER:	G. J. Todaro	Chief, LVC	LVC NCI
	C. Sherr	Public Health Officer	LVC NCI

COOPERATING UNITS (if any)
R. Lemons, Geneticist, George Washington University, W. Nash, Adjunct Prof. of Genetics, George Washington University, J. Shannon, Microbiologist, American Type Culture Collection, M. Barile, Microbiologist, Food and Drug

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5.	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The construction of somatic cell hybrids between cell lines derived from several different mammalian species has permitted the detection, characterization and genetic localization of a number of cellular genes which participate in endogenous type C virus expression. The genes fall into three classes viz. (1) endogenous retravirus DNA sequences in normal DNA, (2) chromosomal integration sites for proviral DNA, (3) restriction genes which delimit replication of retravirus in heterologous cell types. Viral and genetic characterization of two loci Bvr-1 and Bevi is advanced and the analysis of eight additional genes is in progress. Unequivocal genetic signatures of human and mouse cell lines have been developed using allozyme genotypes for purposes of cell contamination and transformation monitors. Quantitative analysis of viral expression and kinetics of synthesis of cell surface p30 and gp70 on retravirus infected cells has been achieved.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. The construction of genetic maps of cat, mouse, and man with respect to genes which participate in neoplastic transformation and related phenomena. The gene classes under study include: (a) retravirus associated genes including endogenous sequences, integration sites, receptor structural genes and restriction genes, (b) genes associated with cell culture correlates to malignancy.

2. Genetic analysis of various genes and alleles of these which actively participate in viral expression and spontaneous transformation. These experiments include (a) cis-trans tests for assessment of positive (inducible) vs. negative (repressible) control mechanisms, (b) complementation matrix construction within species using polymorphic loci and between species taking advantage of evolutionary divergence and fixation of both cellular regulatory genes in parallel with the viral gene targets of the cellular control, (c) placement of various gene types in same cell or in mutant configurations in an attempt to genetically dissect the developmental sequence of gene action which proceeds to virus expression and neoplastic transformation.

3. Use of inherent genetic variation in human and mouse cells to provide genetic signatures or markers for cells studied in cell culture laboratories. By using allelic isozyme (allozyme) variation in different isolates of human or mouse cells, a genetic footprint of cells can be derived for cell contamination monitors. The same technology is applied to detection of mycoplasma infections.

4. Genetic control of cell surface antigens (ubiquitous and differentiative) in normal and leukemia cells. This analysis includes detection and characterization of surface antigens, detection of participating genes in appropriate cell hybrids, assessment of coordinate control of expression and/or extinction under different genetic conditions.

Methods Employed:

The following techniques are used: (a) radioimmunoassay for viral proteins and double labeling for detection of cell surface antigens, (b) lactoperoxidase labeling of cell surfaces with radio-iodine, (c) starch gel and polyacrylamide gel electrophoresis (PAGE), and isoelectric focusing, (d) in vitro radioisotope assay of RNA-dependent DNA polymerase as a viral marker, (e) somatic cell hybridization and various cell culture procedures, (f) chemical mutagenesis of cells and selective procedures for acquisition of selective transformed cell lines, (g) cytological chromosome banding procedures, (h) molecular hybridization on cellulose nitrate filters, (i) in situ hybridization on metaphase chromosomes.

Major Findings:

Two feline chromosomal loci have been studied in detail. The first is Bvr-1 which restricts the production of B-tropic MuLV in cat x mouse hybrids. The restriction occurs late in viral assembly since budding viruses and antigens are apparent on cell surface of restricted cells. Bvr-1 has been mapped to the feline X-chromosome where it is syntenic with Hprt, Gpd and Pgk structural genes for cellular enzymes. The restriction is reversible and does not interfere with gag region polyprotein processing. Bvr-1 gene action is pleiotropic insofar as it restricts the IdU mediated induction of xenotropic MuLV (BALB virus: 2) but not the N-tropic MuLV. Pleiotropic restriction of B-MuLV and X-MuLV but not N-MuLV indicates that the targets of Bvr-1 in B-MuLV and X-MuLV are similar but distinct from those in N-MuLV.

The second feline gene is characteristic of the integration site of FeLV. FeLV infected cat mouse hybrids which are segregant for feline chromosomes yield clones which are either positive or negative for FeLV and FeLV-p27 expression. Preliminary evidence suggests that the endogeous FeLV and RD114 DNA sequences are concordantly inherited in the same hybrids. Discordance of FeLV production and over 20 gene-enzyme markers have been obtained in highly segregant colonies.

A human locus Bevi has been detected in mouse x human and hamster x human hybrid cells. Bevi is required for infection and replication of baboon endogenous virus (M7). The genetic and virological characteristics of Bevi suggest that it may be the chromosomal integration site of M7. Bevi has been mapped to human chromsome 6 where it is syntenic with three gene-enzyme systems, ME, PGM_3, and SOD_2.

The electrophoretic resolution of seven relatively polymorphic human gene-enzyme systems expressed in tissue culture cells can be used as a sensitive genetic monitor for intraspecific cell contamination. The gene-enzyme systems include ADA, PGM_1, PGM_3, G6PD, PGD, PEPD, and ESD. An identical genotype at each of the same allozyme loci provides a 95 percent (or greater) confidence estimate of the identity of two cultured lines, on the basis of the allelic frequencies of the seven enzyme loci in natural populations and in populations of independently derived cultured cells. Of 27 commonly used human cell lines examined, only one of 351 pairwise comparisions proved genetically indistinguishable.

The number of group and interspecies virus associated antigenic determinants expressed on the surface of retravirus infected feline and murine cell was estimated. Both p30 and gp70 are expressed on cell surfaces of feline and murine cells in amounts near half of the total number of virus associated cell surface antigens. The actual number varies between isolates within and between species but the maximum amounts were 1.9×10^5 p30 and $7.5.10^5$ gp70 determinants per cell.

The kinetics of synthesis of cell surface p30 was estimated at between 6 and 8 hours with a much longer lived nRNA.

Significance to Biomedical Research and the Program of the Institute:

The analysis of the metabolism of leukosis virus infection and its relationship to antigen assembly provides important information about the course of cell modification as the result of tumor virus infection and expression. The understanding of the virus and host genetic machinery active in a malignant cell is necessary for any meaningful attempt to correct or destroy cancerous tissue. The resolution of surface antigens on the virus-infected cell surface and the comparative immunogenicity of cell surface antigens are of importance in the evaluation of immunotherapeutic procedures for tumor cell protection and removal. The parasexual genetic analysis of gene action following neoplastic transformation is an important approach to the understanding of genetic regulation of cell division in normal and malignant mammalian cells.

Publications:

1. O'Brien, S.J., Boone, C.W., Simonson, J.M. and Austin, F.: The paired label assay for cell surface antigens. Tissue Culture Association Manual. 2: 423-427, 1976.

2. MacIntrye, R.J. and O'Brien, S.J.: Interacting gene enzyme systems in Drosophila. Ann. Rev. of Genetics, Vol. 10: 281-318, 1976.

3. O'Brien, S.J.: Bvr-1: A C-type RNA virus restriction locus in the feline cellular genome: Identification, location and phenotypic characterization in cat x mouse somatic cell hybrids. Proc. Natl. Acad. Sci. 73: 4618-4622, 1976.

4. O'Brien, S.J., Simonson, J.M. and Boone, C.W.: Expression of viral structural proteins on murine cell surface in association with the production of murine leukemia virus. J. Gen. Virol. 33: 355-360, 1976.

5. O'Brien, S.J.: Isozymes and allozymes as genetic footprints of cultured cells. In Petricianni, J. (Ed.): Proceedings of the FDA Conference on Cell Substrates for Vaccine Production, pp. 46-59n. 1976.

6. O'Brien, S.J., Kleiner, G., Olson, R. and Shannon, J.: Enzyme polymorphisms as genetic signatures in human cell cultures. Science 195: 1345-1348, 1977.

7. Editor: O'Brien, S.J. (Ed.): Isozyme Bulletin. Bethesda, ABC Business Service, 1976, Vol. 9, 1-90 pp.

8. Editor: O'Brien, S.J. (Ed.): Isozyme Bulletin. Bethesda, ABC Business Service, 1977, Vol. 10, 1-90 pp.

9. O'Brien, S.J. and MacIntryre, R.J.: Biochemistry and genetics of enzymes and specific proteins in Drosophila melanogaster. In Wright, T.R.F. and Ashburner, M. (Ed.): Genetics and Biology of Drosophila, New York, Academic Press, 1976, Vol. 11 (In press).

10. O'Brien, S.J. and Boone, C.W.: Expression of feline leukemia virus antigens on cat lymphoma cells: Kinetics of biosynthesis. J. Gen. Virol., 1977 (In press).

11. Lemons, R.S., O'Brien, S.J. and Sherr, C.S.: A new genetic locus Bevi on human chromosome six which controls the replication of baboon type-C virus in human cells. Cell (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04903-01-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)
To Develop Methods of Immunodiagnosis and Immunotherapy of Tumors in Animal Models with Virus-Augmented Tumor Associated Antigens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	N. Takeichi, M.D., Ph.D.	Expert Consultant	LVC	NCI
OTHER:	C.W. Boone, M.D., Ph.D.	Medical Officer	LVC	NCI
	T. Oikawa, M.D.	Visiting Fellow	LVC	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The radioisotopic footpad assay was found to be sensitive enough for detecting tumor associated antigens in non-virus-induced tumors of mice and rats.

2. Human delayed hypersensitivity reactions to BCG and/or Varidase was adoptively transferred to X-irradiated mice with this assay.

3. Influenza virus-augmented tumor associated antigens were successful for preventing metastasis and regrowth of tumors in mice after excision of tumors.

4. The specific marker for rat thymus cells was discovered using the rosette formation test with guinea pig erythrocytes.

5. The virus-augmented tumor associated antigens induced positive footpad reactions in histoincompatible tumor-bearing mice.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. Improvement and development of a radioisotopic footpad assay in mice as part of the development of specific anti-tumor reactions for a bioassay of immune status in human cancer patients.

2. Immunotherapy of tumors in mice with virus-augmented tumor associated antigens.

3. Analysis of the mechanism of virus-augmentation in infected tumor cells.

4. Immunologic study of progressor and regressor Moloney sarcoma viruses.

5. Study of specific markers for thymus cells and T-lymphocytes in the rat.

Methods Employed:

A radioisotopic footpad assay for measuring anti-tumor cell-mediated immunity. Tumor-graft rejection assay. Membrane immunofluoresence and antibody-dependent cytotoxicity assays for detecting tumor associated antigens. Focus formation and XC assays for various murine leukemia and sarcoma viruses. Biochemical procedures are related to the isolation of tumor associated antigens from cultured cell lines infected with influenza virus and Moloney sarcoma virus. Rosette formation test for detecting thymus dependent lymphocytes in the rat.

Major Findings:

Objective 1

a. The radioisotopic footpad assay was found to be sensitive enough for detecting tumor associated in non-virus-induced tumors of mice and rats. Cross reacting tumor associated antigens in methylcholanthrene-induced squamous cell carcinomas of mice were detected by this assay.

b. Human delayed hypersensitivity reactions to living BCG and/or Varidase was adoptively transferred to X-irradiated mice with this assay. The intensity of the footpad reactions to mixtures of test antigens and lymphocytes from human donors correlated with the degree of donor skin test reactivity against the antigen.

c. The anti-tumor immune reaction was adoptively transferred between species by the injection of a mixture of tumor cells and lymphocytes from the tumor-immune or tumor-bearing rats into the footpad of X-irradiated mice.

Objective 2

a. Influenza virus-augmented tumor associated antigens were successful for preventing matastasis and regrowth of tumors in mice after excision of tumors.

b. The antitumor footpad reaction became positive in histoincompatible tumor-bearing mice only when the virus-augmented tumor associated antigens were used.

Objective 3
a. The antigenicity of whole intact tumor cells infected with influenza virus was not increased compared to that of non-infected intact tumor cells, while tumor associated antigens isolated from virus-infected tumor cells showed virus augmentation. This suggests that infection of tumor cells with influenza virus stabilizes the tumor associated antigens against chemical degradation.

Objective 4
a. Recently, Dr. Herberman et al., isolated new stocks of Moloney sarcoma virus which produce progressive tumors in most adult mice. This virus induced lower levels of cell-mediated immunity and produced low antigenic primary tumors.

Objective 5
a. Although rats have been widely used for cancer research, specific markers for thymus cells and T-lymphocytes in the rat have not been established. It was discovered that 20 to 80% of rat thymus cells formed spontaneous rosettes with guinea pig erythrocytes. X-irradiation or treatment with cortisone significantly suppressed the number of rosette-forming cells.

Significance to Biomedical Research and the Program of the Institute:

Objective 1
The local adoptive transfer of the antitumor cell-mediated immunity between species by the radioisotopic footpad assay provides an unprecendented in vivo assay system for the study of immunological phenomena. This assay can detect cross reacting antigens between different types of tumors and monitor immune status of the tumor-bearing host.

Objective 2
The virus-augmented tumor associated antigens will retain strong antigenicity and therefore can be applied for immunotherapy of tumors in the animal model.

Objective 3
Analysis of the mechanism of virus-augmentation will develop purification of tumor associated antigens with specificity and high antigenicity.

Objective 4
By study of progressor and regressor Moloney sarcoma viruses, control mechanism of tumor growth produced by tumor viruses can be clarified.

Objective 5

Discovery of the rat thymus specific marker will be very useful to cancer researchers who investigate immunological phenomena in the rat.

Proposed Course:

1. The applicability of the radioisotopic footpad assay to the bioassay of antitumor immunity in human cancer patients appears feasible. Peripheral blood lymphocytes from cancer patients, mixed with autochthonous allogeneic cancer cells will be inoculated into the footpad of X-irradiated mice or nude mice and resulting delayed hypersensitivity reaction can be measured by this assay.

2. Studies will continue on the prevention of tumor-metastasis and regrowth in the host after conventional treatment by immunizing with virus-augmented tumor associated antigens.

3. The mechanism of virus-augmentation will be analyzed by comparative studies between cytopathogenic viruses (influenza, vesicular stomatitis, etc.) and presistent infectious viruses (murine leukemia, measles, vaccinia etc.).

4. Immunological regression of spontaneously or chemically-induced tumors infected with regressor Moloney sarcoma virus will be studied.

5. Cell origin of thymomas and lymphomas in the rat will be studied using the rat thymus specific marker.

Publications

1. Takeichi, N., Economou, G.C. and Boone, C.W.: Accelerated regeneration of trypsin-treated surface antigens of SV40 transformed BALB/3T3 cells induced by X-irradiation. Cancer Res., 36: 1258-1262, 1976.

2. Boone, C.W., Takeichi, N., Paranjpe, M.S. and Gilden, R.: Vasoformative sarcomas arising from BALB/3T3 cells attached to solid substrates. Cancer Res., 36: 94-101, 1976.

3. Takeichi, N., Economou, G.C. and Boone, C.W.: Local adoptive transfer of antitumor cellular immunity to xenogeneic animals studied with a radioisotopic footpad assay. J. Natl. Cancer Inst., 56: 131-134, 1976.

4. Yoshida, M.C., Sasaki, M., Takeichi, N. and Boone, C.W.: Karyotypes of vasoformative sarcomas arising from BALB/3T3 cells attached to polycarbonate plates. Cancer Res., 36: 2235-2240, 1976.

5. Paranjpe, M.S., Boone, C.W. and Takeichi, N.: Specific paralysis of the antitumor cellular immune response produced by growing tumors studied with a radioisotopic footpad assay. Annals of the New York Academy of Sciences, 276: 254-259, 1976.

6. Takeichi, N., Boone, C.W. and Klein, E.: Local adoptive transfer to mice of human delayed hypersensitivity reactions by the radioisotope footpad assay. Clin. Exp. Immunol., 26: 310-314, 1976.

7. Takeichi, N., Kodama, T., Kobayashi, H. and Boone, C.W.: Adoptive immunotherapy of Gross Virus producing lymphomas and methylcholanthrene-induced fibrosarcomas in tolerant rats. Cancer Res., 36: 4039-4043, 1976.

8. Economou, G.C., Takeichi, N., and Boone, C.W.: Common tumor rejection antigens in methylcholanthrene-induced squamous cell carcinomas of mice detected by tumor protection and a radioisotopic footpad assay. Cancer Res., 37: 37-41, 1976.

9. Takeichi, N. and Boone, C.W.: Spontaneous rosette formation of rat thymus cells with guinea pig erythrocytes. Cellular Immunol., 27: 52-59, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01-CP-04916-01-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Organization and Evolution of Endogenous Mammalian Type C Viral Genes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T. I. Bonner	Expert	LVC, NCI
OTHER:	R. E. Benveniste	Microbiologist	LVC, NCI
	G. J. Todaro	Medical Officer and Chief	LVC, NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Techniques are being developed to obtain DNA probes specific to various regions of the genome of the endogenous baboon virus. These probes should allow DNA hybridization measurements to determine which viral genes are most conserved both between species and within the multiple copies of viral related sequence in the baboon. Probes specific to the most conserved regions of the viral genome may provide improved sensitivity for endogenous human viral sequences.

The virus PP-REC, thought to be a recombinant virus containing baboon, mink, mouse and rat viral information, is also being studied in order to determine which portions of the viral genome are specified by each species of viral information.

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04916-01-LVC

Project Description

Objectives:

To study evolution of mammalian endogenous type C viral genes and their organization within the mammalian genome.

Methods Employed:

DNA probes complementary to varying lengths of viral sequence starting at the 3' end of the virus are generated. Hybridization of probes of varying length to DNA of other species allows mapping of the regions of homology of viral genes even when virus has been isolated from only one of the two species being compared. Probes specific to certain regions of the viral genome are also prepared by hybridization to RNA from related viruses.

Major Findings:

Probes representing the 3'-ended quarter to half of the baboon viral genome have been prepared. Hybridization measurements are in progress.

Significance to Biomedical Research and the Program of the Institute:

Our studies should provide more detailed information on the evolution of endogenous viral genes. The most conserved regions of the baboon viral genome may provide a probe of improved sensitivity for the assay of endogenous human viral sequences.

Proposed Course:

Increase the length of 3' initiated probes and continue the measurements in progress.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04917-01-LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cellular Mechanisms of Carcinogenesis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Devi Vembu, Ph.D.	Visiting Fellow	LVC	NCI
OTHER:	Charles Boone	Medical Officer	LVC	NCI
	Lance Liota	Resident Pathologist	LP	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Cloning of endothelial cells from mouse subcutaneous connective tissue is in process, with the aim of characterizing the type of tumors they produce. The collagenase activity of tumor cell lines of defined tumorigenicity and invasivity is being determined. Karyological analysis of adult mouse fibroblasts undergoing spontaneous neoplastic transformation is being performed.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To prepare cloned lines of endothelial cells from subcutaneous granulation tissue in mice, carry the lines until spontaneous neoplastic transformation *in vitro* occurs and characterize the types of tumors the lines produce.

2. To examine tumor cell lines of defined tumorigenicity and invasiveness for collagenase activity.

3. To determine ways of testing the chromosome instability theory of carcinogenesis.

Methods Employed:

Induction of subcutaneous granulation tissue by insertion of nylon sponges, identification of endothelial cells with fluoresence-labeled antibody specific for type IV collagen, standard cytogenetic techniques.

Major Findings:

1. During the fourth week after implantation of nylon wool subcutaneously in mice, intensive ingrowth of capillary endothelial cells occurs. The nylon sponges can then be removed and the endothelial cells obtained by treating with collagenase and trypsin. Cloning procedures to isolate endothelial cells are in progress.

2. Collagen can be biosythetically labeled *in vitro* with 3H-proline. The collagenolytic activity of tumor cells is being determined by inoculating mixtures of labeled collagen and tumor cells at various organ sites in the mouse and taking tail-blood samples for levels of radioactively labeled soluble collagen products.

3. Lines of adult mouse connective tissue are being routinely explanted and subcultured on fixed schedules, then tested for tumorigenicity in syngeneic mice. Karyological changes are being monitored during this process. Heteroploid cells from tumors will be characterized for their "chromosome dosage profile", i.e., the number of each type of chromosome present.

Significance of Biomedial Research and the Program and the Institute:

1. An open controversy exists as to the specific types of tissue cell which most commonly undergoes spontaneous neoplastic transformation *in vitro*. Some authorities contend that the cell is an endothelial cell. Determining the characteristics of tumors produced from cloned endothelial cells will help to settle this question.

2. The properties of tumor cells that give them invasive capability are important to identify. Collegenolytic activity may be one of these.

3. The hypothesis that chromosomal instability can give rise to high chromosomal mutation rates from which variants arise that are independent of host regulatory controls is one favored by many investigators. Recent advances in cytogenetics now make this hypothesis testable.

Proposed Course:

Work in progress will occupy the next two years. Following this, the interaction of neoplastic connective tissue cells with collagen will be explored.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04918-01 LVC

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Analysis of the Mechanism of Virus Augmentation by Influenza Virus.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Tsuneyuke Oikawa, M.D.	Visiting Fellow	LVC	NCI
OTHER:	Charles W. Boone	Medical Officer	LVC	NCI
	Noritoshi Takeichi	Expert Consultant	LVC	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH Office of the Associate Director, ~~Viral Oncology Program~~
Laboratory of Viral Carcinogenesis

SECTION
Cell Biology Section

INSTITUTE AND LOCATION Division of Cancer Cause & Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1. The immunogenicity of tumor crude membranes is markedly augmented if the tumor cells are first infected with influenza virus, as detected by a radioisotopic footpad delayed hypersensitivity in mice. (Virus-augmentation). Virus-augmentation was observed both in mouse systems and rat systems.

Tumor protection using crude membranes from tumor cells infected with influenza virus was successful if the tumor was highly antigenic, whereas crude membranes from non-infected tumor cells did not inhibit tumor growth at all.

2. Recent reports have shown that an important relationship exists between tumor associated antigens (TAA) and major histocompatibility (MHC) antigens. Virus-augmentation was abrogated by adding anti-influenza virus serum to virus-infected tumor cell membranes. Studies on the abrogating effect of antisera against tumor antigen and against the MHC are in progress.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To demonstrate virus-augmentation in various kinds of rat and mouse tumor systems.

2. To detect changes in the number of tumor associated antigens and major histocompatibility antigens after influenza virus infection.

3. To determine the immunological relationship between the influenza virus particle, tumor associated antigens, and histocompatibility antigens on the tumor cell surface infected with influenza virus.

Methods Employed:

Tumor rejection assay, delayed hypersensitivity reaction against tumor antigens by radioisotopic footpad assay, complement dependent cytotoxicity assay.

Major Findings:

1. The immunogenicity of tumor crude membranes is markedly augmented if the tumor cells are first infected with influenza virus, as detected by a radioisotopic footpad delayed hypersensitivity in mice (virus-augmentation). Virus-augmentation was observed both in mouse systems and rat systems.

Tumor protection using crude membranes from tumor cells infected with influenza virus was successful if the tumor was highly antigenic, whereas crude membranes from non-infected tumor cells did not inhibit tumor growth at all.

2. The mechanism of virus-augmentation is still under investigation. One possible explanation is that virus infection increases the number of tumor associated antigens on the cell surface. This possibility is now being pursued using complement dependent cytotoxicity assay.

3. Recent reports have shown that an important relationship exists between tumor associated antigens (TAA) and major histocompatibility (MHC) antigens. Virus-augmentation was abrogated by adding anti-influenza virus serum to the virus-infected tumor cell membrane. Studies on abrogating effect of anti-sera against tumor antigen and against the MHC are in progress.

Significance to Biomedical Research and Program of the Institute:

Virus-augmented tumor membrane preparations may be useful as skin test antigens for evaluating the immune status of cancer patients. Studies of virus-augmentation system may also shed light on the relationship between tumor associated antigens and histocompatibility antigens.

Publications:

1. Hosokawa, M., Oikawa, T., Nakayama, M., Sendo, F., Gotohda, E., Kodama, T. and Kobayashi, H.: Inhibition of syngeneic tumor growth in rats immunized with allogeneic skin grafts. Inter. J. Cancer, 18: 369-374, 1976.

2. Gotohda, E., Kawamura, T., Sendo, F., Nakayama, M., Akiyama, J., Oikawa, T., Hosokawa, M., Kodama, T. and Kobayashi, H.: Effect of combination treatment with cyclophosphamide and non-specific passive immunization on a transplantable tumor in WKA rats. Cancer Res., 36: 2119-2123, 1976.

3. Oikawa, T., Hosokawa, M., Imamura, M., Sendo, F., Nakayama, M., Gotohda, E., Kodama, T. and Kobayashi, H.: Anti-tumor immunity by transferring allogeneic blood in rat. Clin. Exp. Immunol. (In press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04919-01-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Role of Type C Viruses in Human Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. A. Sherwin	Research Associate	LVC, NCI
OTHER:	G. J. Todaro	Medical Officer and Chief	LVC, NCI
	R. E. Benveniste	Microbiologist	LVC, NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Viral Leukemia and Lymphoma Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Type C viruses have been causally linked to leukemia and sarcoma in a variety of vertebrate species including at least one primate. However, the role of these viruses in human malignancy remains unclear. We have conducted experimentation in several areas to further elucidate this problem. A wide range of human tumor specimens have been analyzed for the presence of infectious type C virus by techniques of in vitro cocultivation, thus far with negative results. Moreover, many of the same tissues were analyzed by nucleic acid hybridization for the presence of gene sequences related to those of the endogenous type C virus of baboons, the only endogenous virus isolated from primates. There is, at present, no firm evidence that this virus has been horizontally transmitted to man, thereby resulting in malignancy. Finally, a series of experiments were conducted to determine whether antibody-independent, complement-mediated viral lysis might prevent the spread or release of type C viruses in primates. However, such virolytic activity was detected in those primate species which do, in fact, release and horizontally transmit type C viruses.

1830

PHS-6040
(Rev. 10-76)

Project No. Z01-CP-04919-01-LVC

Project Description

Objectives:

To determine the role of type C viruses in human malignancy.

Methods:

Fresh human tumor material was subjected to in vitro cocultivation in an attempt to isolate infectious type C virus. Similar or identical specimens were analyzed for the presence of nucleic acid sequences related to the baboon type C virus by DNA:DNA hybridization techniques. Complement-mediated viral lysis was studied by means of the reverse transcriptase release assay (preincubated mixtures of serum and virus are assayed for reverse transcriptase activity).

Major Findings:

The above experiments have failed to reveal the presence of either endogenous type C virus or horizontally acquired baboon type C virus in any of the human tumor specimens examined to date. Moreover, studies on complement-mediated viral lysis failed to support the proposition that such virolytic activity blocks the release or horizontal transmission of type C virus in primates, including those with this activity in their sera.

Significance to Biomedical Research and the Program of the Institute:

These experiments have addressed themselves to the centrally important question as to whether type C viruses are causally linked to human malignancy.

Proposed Course:

Experiments are now in progress which are attempting to isolate endogenous type C virus from human tumor material by means of virus infection and activation. Additional human tumor specimens are being analyzed for the presence of virogene sequences related to those of baboon type C virus.

Publications:

None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01-CP-04920-01-LVC

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Analysis of Malignant Cell-surface Antigens and the Effects of a Preparation of Streptococcus pyogenes (OK-432) on Host Immune Response

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Suzuki	Guest Worker	LVC NCI
Other:	T. Aoki	Head, Immunology Section	LVC NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH Office of the Associate Director, Viral Oncology Program
Laboratory of Viral Carcinogenesis

SECTION
Immunology Section

INSTITUTE AND LOCATION Division of Cancer Cause and Prevention
NCI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Analysis of Moloney murine sarcoma virus (M-MuSV) and Moloney murine leukemia virus (M-MuLV)-associated surface antigens and host immune responses to them.

Studies of effects of OK-432 on immune response of normal mice and on the growth of Moloney murine sarcoma virus (M-MuSV)-induced primary tumors in mice of various strains.

PHS-6040
(Rev. 10-76)

Project Description

Objectives:

1. To distinguish the tumor-specific cell surface antigens acquired by malignant transformation from the surface antigens of nonmalignant cells associated with type C virus, to characterize these antigens, and to analyze immune response to the tumor cell surface antigens.

2. To examine the relationship between the immunotherapeutic effect of OK-432 and the nonspecific as well as specific immune responses.

Methods Employed:

1. & 2. Radioimmuno-precipitate-SDS polyacrylamide gel electrophoresis (PAGE) analysis, radioimmunoassay (RIA) using intact M-MuLV, virus neutralization tests, and measurement of tumor growth.

Major Findings:

1. Humoral antibody formation of BALB/c mice was analyzed after inoculation of M-MuSV. By M-MuLV neutralization tests, humoral antibody was detected at low titers two weeks after virus-inoculation. After this period of time, the virus-neutralizing activity decreased in sera from the tumor-progressors, and increased in sera from the tumor-regressors. Cytotoxic activity to M-MuLV-induced BALB/c leukemia (LSTRA) cells was also higher in regressors than in progressors. However, sera from both progressors and regressors showed the equivalent activity to intact M-MuLV detected by RIA. By study of antigens on the surface of LSTRA cells, a virus structural component which possessed new molecular weight larger than 70,000 daltons was demonstrated on LSTRA cells by PAGE analysis.

2. OK-432 treatment significantly inhibited the growth of M-MuSV-induced tumors in BALB/c, C3H/He, and AKR mice. The sizes of M-MuSV-induced tumors in both OK-432-treated and PBS-treated BALB/c mice were corresponded to M-MuLV neutralization activity titers for their sera.

Significance of Biomedical Research and the Program of the Institute:

1. Our findings showed that the specific immune response to viral envelope antigen, which was detected by virus neutralization antibody but not by RIA with intact M-MuLV, affects the tumor growth in mice. This phenomenon was also confirmed by the same results observed in OK-432-treated regressors.

Newly detected antigen on cell surface of LSTRA cells would provide a first step to further survey in detail the relationship between type C virus and tumor cell-surface antigens in mice.

2. The immunotherapeutic effect of OK-432 was demonstrated on M-MuSV-induced tumor in several strains of mice. Our results indicate that OK-432 enhanced the immune response in our experimental system.

Proposed Course:

1. It is planned to analyze the specificity of regressor serum and to isolate and characterize the immunological and biological properties of new antigen on the cell surface of LSTRA cells.

2. Further studies are planned of the immunotherapeutic effect of OK-432.

Publications:

Osugi, Y., Suzuki, S. and Takagaki, Y.: Antitumor and immunosuppressive effects of mycophenolic acid derivatives. Cancer Research 36: 2923-2927, 1976.

Kai, K., Ikeda, H., Yuasa, Y., Suzuki, S. and Odaka, T.: Mouse strain resistant to N-, B-, and NB-tropic murine leukemia viruses. J. Virology 20: 436-440, 1976.

C. 1. COLLABORATIVE RESEARCH BRANCH
July 1, 1976 - September 30, 1977

a. Branch Summary

The Collaborative Research Branch of the Viral Oncology Program, Division of Cancer Cause and Prevention, was established to participate in the planning, development and scientific administration of a program of collaborative research conducted within the Virus Cancer Program on viruses as etiologic agents of cancer in man and animals and on the control of tumor viruses and/or their induced diseases.

The Breast Cancer Virus Studies Section manages contracts conducting research on viruses as a cause of mammary carcinoma in animals and the elucidation of their role in breast cancer in humans with emphasis on the study of virus-like activity demonstrable in human case materials and on the reproduction, characterization, composition and cellular transformations associated with viruses known or suspected to cause mammary gland carcinomas. The Clinical Studies Section manages contracts conducting research (a) to provide evidence for viral associations with neoplasia in human cohorts, (b) to investigate host factors in humans and animals which may modify or otherwise influence susceptibility to infection or the development of neoplasia following infection, (c) to develop methods which may be applicable in prognosis or diagnosis of malignancies, and (c) to investigate methods which may be effective for the control of infection or development of neoplasia in animals and humans. The Cocarcinogenesis Studies Section manages contracts conducting research on the interaction of viruses with chemical, physical and biological environmental agents as cofactors causing neoplastic transformation of mammalian cells, emphasizing: (a) development and standardization of cell-virus systems for study; (b) identification of the interactions which result in transformation; (c) determination of the mechanisms involved; and (d) definition of methods for prevention or control of induction of transformation. The DNA Virus Studies Section manages contracts conducting research on viruses with a DNA core which are known or suspected to be involved in the induction of malignant transformation of animal and human cells and supports studies on (a) elucidation of the role of viruses of this category in the induction of neoplastic disease, (b) their characterization and biological activity, (c) intracellular relationships established with cellular components, (d) virus genome expression and intracellular control, (e) mechanisms of reproduction and induction of neoplasia, and (f) inhibition of replication and cell transformation. The RNA Virus Studies Section manages contracts conducting research on viruses with a ribonucleic acid core which are known or suspected to induce malignant transformation of animal and human cells with primary emphasis upon agents which exhibit characteristics of viruses known to induce leukemia, lymphoma, sarcoma and related diseases in laboratory animals, and supports studies in (a) the detection in case material of activities or components characteristic of viruses of this nature, (b) virus isolation and characterization, (c) virus replicative process, (d) intracellular relationship between virion and cellular components, (e) virus genome expression and intracellular control, (f) mechanisms of virus-induced cell transformation to malignancy, and (g) inhibition of replication and cell transformation. The Office of the Chief coordinates the research activities within the collaborative research

area with respect to the objectives of the Virus Cancer Program. The Chief directs the activities within the Sections with the assistance of the Associate Chief and reports to the Associate Director for the Viral Oncology Program.

The program of research on the role of viruses in mammary carcinogenesis has been restricted by the availability of only one animal system for intensive study, that of the type B RNA mouse mammary tumor virus (MMTV) in in mice. At present, only one other carcinoma-producing virus has been recognized; this is the Lucke herpesvirus of the leopard frog, which appears to be the etiologic agent for renal carcinoma in that species. In view of the desirability to define other experimental animal systems for viral studies on mammary gland carcinogenesis, two new projects were initiated during the past year. One project is investigating the mammary cancers of cats and the other is concentrating on these cancers in dogs for evidence of a viral association.

Primary cultures of canine mammary carcinomas contained mixed cell populations. Although some tumor biopsies and tissue culture materials contained virus-like particles observed by electron microscopy, no stages of virus reproduction were detected. Different procedures known to activate repressed virus genetic information were applied to tumor cell cultures. The treated cultures yielded no significant evidence of reverse transcriptase activity and no virus activity detected by XC or KC cell responses.

Several mammary carcinomas of the cat contained intracisternal type A particles. Some tumors contained antigens which reacted with a polyvalent antiserum prepared against MMTV. Recently it was found that antiserum monospecific for the 34,000 molecular weight glycoprotein (gp34) of MMTV will react with cells containing a large number of the intracisternal type A particles. The specificity of this reaction has yet to be evaluated. Reverse transcriptase activity was detected in a few feline mammary tumors, but no special significance can be ascribed to this observation, since type C virus infection is common in cats.

Several cell lines produce MMTV. Production by mouse cells is associated with the presence of "dome" formations in the cultures. Producing cultures are selected for consistency of production of MMTV at high levels and the absence of type C virus group specific antigen. The polypeptide components of the virus have been identified. Differences were observed in the composition of the polypeptides obtained from virus recovered from mouse milk as compared to recoveries from tumor cell cultures. The availability of larger amounts of MMTV from cell cultures than possible from mouse milk and of antisera to purified virion polypeptides has provided increased opportunity to study the disease process in mice and to probe for related components expressed in mammary cancers in humans and in other animals.

A single dominant gene (Mtv-1) in the C3Hf mouse strain controls the release into milk of MMTV-L, the low oncogenic variant of mouse mammary tumor virus. The C57BL mouse strain harbors a single epistatic gene (Imv) which can inhibit the release of MMTV-L, although it has no effect on production of the more oncogenic MMTV-P variant in the GR strain. The genetic locus, Mtv-2, which is responsible for release of high levels MMTV-P and early hormone dependent mammary tumors, consists of about eight tandem copies of MMTV DNA.

Vaccination of mice with a virion protein fraction rich in glycoprotein, gp52, resulted in a slight delay of mammary tumor development. A degree of resistance to tumor transplants was obtained when low doses of vaccine were used with an adjuvant. Higher doses of MMTV protein accelerated tumor growth. This appeared to be associated with poor cellular immune reactivity to the virus.

Mice of the BALB/c strain do not release infectious MMTV into milk. However, their lymphocytes responded to MMTV from strain RIII mice and from C3H mouse tumor cell cultures, but the reactivities to virus from the two sources were not identical. Tests to localize the antigen conferring this sensitivity to BALB/c lymphocytes detected its presence in splenocytes but not in thymocytes. This suggests partial endogenous virus genetic expression in the splenocytes in the absence of virion production.

There appear to be consistent changes in the nuclear, nucleolar, mitochondrial, cytoplasmic and surface ultrastructure of neoplastic human mammary cancer cells as compared to normal cells. Primary mammary epithelial cells grow readily on myoepithelial cell monolayers. Apparently, myoepithelial cells produce a diffusible factor which promotes epithelial cell growth. An established human mammary cell line (MCF-7) is a mixture of predominantly small cells with a large cell component which has a lower nucleus to cytoplasm ratio. The MCF-7 cell line previously was observed to release virus particles in small numbers, but production has not been consistent. Virus-specific complementary DNA was prepared by the endogenous reverse transcriptase reaction. Used as a probe, this cDNA detected virus RNA in both virus-producing and non-producing cells, but less virus RNA was present in the non-producing cells. Treatment of MCF-7 cells with different hormones and chemical inducing agents did not augment virus release. Antigens reactive with antisera to MMTV and MMTV-related RNA was detected in MCF-7 cells. The stringency of the nucleic acid hybridizable MCF-7 RNA has extensive sequence homology with the MMTV cDNA probe used.

Antisera to the major glycoprotein component of MMTV were used to probe for related antigenic determinants in human milk specimens fractionated by zonal centrifugation. Some positive findings were obtained with pooled milks, but the negative results with individual specimens suggested very low concentrations or infrequent occurrence of the reactive component. The reverse transcriptase activity detected in human milk shares some, but not all, of its properties with the MMTV enzyme. Extracts prepared from human mammary gland tissue were tested in one laboratory for the presence of antigens which would compete in radioimmune precipitation of intact labeled MMTV by antisera specific for MMTV and its gp52 and gp36 glycoprotein components. No competing antigens were detected by this sensitive procedure. Data acquired in another laboratory indicated that the leukocytes from some breast cancer patients react against the gp55 of MMTV from RIII strain mice as well as against some autologous and homologous breast cancer tissues. Antiserum prepared in rabbits against gp55 blocked the response in vitro of breast cancer patients' leukocytes against gp55 and against breast cancer tissues. In contrast to the reactivity of the leukocytes against the RIII MMTV gp55, few positive reactions were obtained in tests against gp50 from A mouse strain MMTV. The nature of the cross-reacting antigens present in human mammary cancer tissues is not known.

Studies on the role of DNA viruses in neoplasia included the Epstein Barr virus (EBV); Herpes simplex viruses types 1 and 2 (HSV-1; HSV-2); Cytomegalovirus (CMV); Herpesvirus saimiri (HVS); Herpesvirus ateles (HVA); Marek's disease herpesvirus (MDHV); adenoviruses (Ad); human wart virus (HWV); and the BK papovavirus.

Persistent infection with EBV is widespread in humans. Interest in this virus was generated by the consistency of its association with Burkitt's lymphoma in Africans. A close relationship was established between this virus and anaplastic nasopharyngeal carcinoma (NPC) occurring in humans in all parts of the world. Conclusive evidence was obtained for an etiologic role for EBV in the development of infectious mononucleosis (IM) in young adults.

A number of antigens were defined in B-lymphocytes infected by EBV. Apparently all infected cells contain the virus-specified nuclear antigen, EBNA. A soluble complement-fixing antigen (S antigen) could be extracted from cells which carry the virus. Early antigens (EA), which appear in cells shortly after infection, were observed by immunofluorescense assay to be of two forms, a clumped R form and a diffuse D form. Virus capsid antigens (VCA) develop in the cell cytoplasm and membrane antigens (MA) appear on cell surfaces. The cell exerts controls over virus genetic activity expressed by the presence or absence of these antigens. Considerable attention was given to patient immune responses to the different virus-related antigens throughout primary lymphoma, therapeutically induced remission, and relapse to define the relationship between the virus and the disease process.

Recently, EBNA has been purified, concentrated and characterized. Elution characteristics and immunological evidence suggest EBNA sub-type differences. EBNA binds antibody only in the presence of DNA. Observations on hybrids of mouse fibroblasts with cells from an NPC biopsy implicate chromosome 21 in the maintenance of EBV DNA and EBNA.

The production of EA is a prerequisite for VCA production. However, VCA production is regulated by partly independent intracellular controls. In some lines, EA positive but VCA negative cells synthesized virus DNA, showing existence of a block between DNA and VCA production.

The presence of EBV-specific killer T-cells in the circulation of IM patients was correlated with the presence of anti-VCA IgM antibodies and the absence of antibodies against EBNA. EBV-specific killer T-cells were irregularly isolated from the tumor tissue and regional lymph nodes of BL and NPC patients but not from their peripheral blood. Purified T-cell fractions were obtained by the selective removal of non-specific cytotoxic cells.

Seroepidemiological studies provided evidence suggesting an association between past genital infection with HSV-2 and cervical carcinoma. Subsequent work showed that HSV-2, rendered non-cytolytic by prior exposure to ultraviolet irradiation, could induce transformation of rodent cells in culture. However, such infection activated the expression of endogenous RNA xenotropic virus in the rodent cells, raising a question regarding the role of each virus in the process of transformation.

Examination of mouse cells biochemically or morphologically transformed by irradiated HSV for HSV DNA content detected fragments ranging in size from 28 percent to 3 percent of the virus genome. The upper limit of HSV DNA required for cell transformation is 3 to 4 percent. In one class of transformed cells, no detectable HSV DNA remained after 60 tissue culture passages. Studies on transformation by transfection with fragments of HSV DNA indicated that this approach is feasible for determination of the virus DNA fragment responsible for transformation.

Studies on the incorporation of fucose into HSV transformed hamster and human cells showed a block in the conversion of fucose to fucolipid IV in malignant phenotypes. This block correlated with the cell cycle dependent expression of the early virus-induced nonstructural polypeptide, VP134.

A number of HSV-induced proteins and glycoproteins were isolated and purified. Among these, VP123 possessed antigenic determinants predominantly specific for HSV-1 and VP119 for HSV-2. By radioimmune precipitation tests, type antibody could be detected in the sera of humans with primary HSV-1 or HSV-2 infections. However, a primary HSV-2 infection in individuals previously infected with HSV-1 may raise serum antibodies to the antigens shared by both viruses and no antibodies against the HSV-2 type specific antigens.

Serum from patients with cervical dysplasia or with carcinoma *in situ* modified and suppressed lymphocyte response to two non-specific mitogens, phytohemagglutinin and concanavalin A. Although lymphocyte responses to purified HSV-1 and HSV-2 varied from patient to patient within the same category, the lymphocyte responses of cervical cancer patients appeared to be depressed in comparison with lymphocyte responses of patients with recurrent herpetic lesions.

Lymphocytes from sensitized donors in the presence of the sensitizing antigen release a soluble factor which stimulates the metabolism of the hexose monophosphate shunt in human polymorphonuclear leukocytes. This newly observed reaction is specific for the sensitizing antigen. This assay is being refined for application to the study of patients with cervical carcinoma.

Transformed cells were observed in human embryo lung fibroblasts at the twenty-fourth passage after infection with CMV. EBNA-like nuclear antigens were detected in these cells when the anticomplement immunofluorescence technique was applied using CMV-immune human sera. Screening tests with different CMV-immune human sera demonstrated the nuclear antigens in 6 to 80 percent of the cultured cells.

Two simian herpesviruses cause malignant T-cell lymphomas in animals: HVS, recovered from squirrel monkeys and HVA, isolated from spider monkeys. Some, but not all, owl monkeys infected by these viruses develop a malignant leukoproliferative disease. Both viruses induce malignant lymphoma in rabbits. The cotton-top marmoset is susceptible to HVS-induced lymphoma. Cebus monkeys and Macaque species were not affected.

The immune responses of HVS-infected owl monkeys were followed throughout the course of disease. The development of leukemia in this species did not impair the ability of their peripheral blood lymphocytes to act as effector cells in the assay for antibody dependent cytotoxicity. However, the lymphocytes of diseased animals lost responsiveness to phytohemagglutinin (PHA) and concanavalin A in contrast to the response by lymphocytes from infected but clinically normal owl monkeys. A culture of HVS infected lymphoid cells produced a soluble factor which blocked the response of normal lymphocytes to PHA and inhibited DNA synthesis by several types of cultured cells. The nature of this factor has not been established.

The Marek's disease herpesvirus (MDHV) produces a T-cell lymphoma in chickens. Infection by this virus activates endogenous avian leukosis virus in chickens raising questions regarding the interaction between these two viruses with respect to the resulting disease process.

The MKT-1 avian lymphoblastoid cell line carries the MDHV genome but does not produce this virus. MKT-1 cells were treated with iododeoxyuridine, an inducer of virus expression, and examined by electron microscopy. Both herpesvirus and type C leukosis virus particles were present, but in no instance were both viruses detected in the same cell. On the other hand, the WBC-1 line, cultured from the leukocytes of a chicken inoculated with MKT-1 cells, contained antigens related to both MDHV and avian leukosis virus. Karyotypic analyses of transformed cells from chickens inoculated with MKT-1 cells disclosed a chromosome abnormality in the largest No. 1 chromosome pair.

Chicken embryo cultures produce the p19 polypeptide of avian leukosis virus for a short time after infection with MDHV. This may correlate with the enhancement of type C virus expression previously observed in the tumor tissue of chickens infected with leukosis virus-free MDHV. A correlation was observed between factors which block cell-mediated immunity and the initiation of neoplastic lesions characteristic of Marek's disease.

An intensive investigation was made to determine whether an association exists between adenoviruses (Ads) and human cancer. These viruses induce tumors in hamsters. Homology between the DNAs of Ads of different groups showed that the analysis of tumors with DNA probes prepared from 6 or 7 Ads could determine the presence of DNA for 31 Ad types. The analysis of human tumors was made with Ad transforming genes. The study included lung, colorectal, bladder and pancreatic tumors. No virus DNA was detected.

The human wart virus (HWV) not previously propagated *in vitro*, was shown to multiply in cultured epithelial cells of low passage level. Successful replication appears to depend on culture systems in which the cells will differentiate. Such epithelial cultures were obtained with an adaptation of Freeman's organ culture technique. In successfully infected cultures, new virus antigen was detected 4 to 5 days after infection and reached a maximum on the 8 to 9th day.

BK virus, a member of the papovavirus group of small DNA viruses is widely distributed in humans. Tests showed this virus could induce tumors in hamsters. The BK virus has a longer growth period than other papovaviruses. Slight

differences exist in the size and homology of the DNA's of different isolates related to BK virus. Most of the virus DNA is in the form of supercoiled molecules with the remainder as nicked circular molecules. Early tests detected the presence of the virus genome in some human tumors and in some tumor cell lines. Other investigations on a large number of tumors did not support a significant association with human cancers.

The type C RNA viruses have been shown to cause lymphomas, leukemias or sarcomas in diverse species of animals including the mouse, rat, chicken, hamster, cat, monkey and ape. Virus isolated from bovine leukemia case material produces malignant disease in sheep. Accordingly, considerable effort has been made to determine whether similar viruses afflict humans.

Type C virus genetic information is known to integrate into host cell DNA and to pass to succeeding generations as an endogenous component of genetic inheritance.

The integrated type C virus genome is subject to cellular control of genetic expression and may not be expressed as a protein product or as a complete virion. Therefore, detection requires specifically reactive probes such as related nucleic acid sequences which will hybridize with the sequences of the endogenous virus genome. Simian viruses may provide such probes to detect related viral components in human cancers.

A number of simian viruses containing a reverse transcriptase (RT) have been isolated. An endogenous virus (BaEV) was recovered from baboons. Probes prepared from BaEV detect the presence of related genetic information in tissue cells of some humans. Several new isolates of gibbon ape leukemia virus (GaLV) were obtained. Infected gibbons excrete infectious virus in urine and feces permitting horizontal transmission of infection. Vertical transmission to progeny has not been demonstrated. A type D virus, endogenous in squirrel monkeys, is related but not identical to the Mason-Pfizer monkey virus (M-PMV).

The detection in tumor tissue of RT activity suggests the presence of RNA tumor-virus genetic information. Extracts of 20 human placentas examined contained RT activity. Electron microscope studies detected virus-like particles in the trophoblast layer of 7 of 19 human placentas. The enzyme activity was not neutralized by antisera against the RT of the known primate, feline or murine type C viruses. The virus has not yet been propagated in cell cultures. The RT detected in human breast cancer tissues and the RTs present in the spleens of patients with lymphocytic and with chronic myelogenous leukemia have been purified and partially characterized. These enzymes will be used in radioimmunoassays to determine antigenic relationships to known mammalian virus RTs.

Human leukemia cells were probes for the presence of antigens related to simian sarcoma virus (SSV-1), previously isolated from a woolly monkey. No evidence of antigens related to the p30 or gp45 virus polypeptides was found. However, nucleic acid sequences related to the virus nucleic acid were reported to be present in specimens from some patients with acute myelogenous leukemia. A glycoprotein of 55,000 molecular weight detected in membranes of human

chronic granulocytic leukemia cells appears to be related to a minor antigenic determinant on the gp71 of the Friend murine leukemia virus.

Endogenous type C viruses in cells may be activated by chemical agents such as halogenated pyrimidines. By this means, a virus with RT activity was recovered from strain 13 guinea pigs. Similarly, a new amphotropic virus was isolated from clone 31 of cultured Balb/c 373 cells. This virus may be a product of recombination of simultaneously induced endogenous ecotropic and xenotropic viruses, giving it its expanded host range.

Cancer has been said to be a disease of arrested cell differentiation. Studies have been underway to examine the role of oncogenic RNA virus infection on alteration of cell differentiation. Observations on chicken myeloblasts infected with Rous sarcoma virus showed that the effect of this virus on muscle cell differentiation was mediated through the control of cell-specific functions related to DNA synthesis and cell division rather than through the process of fusion of myoblasts to form myotubes.

Considerable work has been done to characterize the protein products of RNA tumor virus genetic expression. A number of gp70 MuLV envelop glycoproteins was isolated from viruses pruified from sera and excretions of several inbred mouse strains. Tryptic peptide maps identified several classes of related virus envelope proteins. More than one type of gp70 could be isolated from a single mouse strain, depending on the anatomical site or origin. Virus-specified cell surface antigens have been identified which may be important in identifying preleukemic cells and in immunoprophylaxis.

Studies on precursor proteins were conducted to define the gene order in oncogenic type C RNA viruses. These showed that the structural (gag) gene is adjacent to the polymerase (pol) gene in the virus genome. The control of virus gene expression in mice was linked to the differentiation of lymphoid and epithelial cells in some mouse strains. The gp70 and the p30 proteins were not coordinately expressed, and different endogenous virus gp70 proteins were expressed in different organs of the mouse.

Mice may develop a natural immune response to antigens of their endogenous viruses depending on the expression of the antigen in tissues and on host genetic factors. The effectiveness of active immunization of mice with purified viral gp71 envelope protein varied among mouse strains. A protective effect was observed in mice of low leukemia strains. Mice of high leukemia strains suffered enhancement of spontaneous disease following active immunization, but this was circumvented by passive immunization with heterologous anti-gp71 serum. Passive immunization with antisera against the interspecies antigens of leukemia viruses also had a protective effect against virus challenge.

Investigations on viral RT yielded definition of conditions allowing transcription *in vitro* of full-length DNA copies of the RNA virus genome. Transfection experiments showed that these DNA copies were biologically active. The transfer RNA requirement for transcription of RNA by RT varies among the viruses from different species. The beta component of RT is active in tRNA binding. A terminal redundancy between the 3' and the 5' ends of

virus RNA was determined. This may be important in jumping from the 5' to the 3' end during transcription.

Electron microscope techniques permitted visualization of the structure of the type C virus genome. This structure is similar for the genomes of all the mammalian type C viruses examined and possibly may include the avian viruses. A homologous gag region exists at the 5' end of related murine sarcoma and leukemia viruses. A short, nonhomologous sequence in the middle region of the sarcoma virus RNA possibly represents the sarc gene region. The observation of circular heteroduplexes may provide a clue to the mechanism of transcription by RT.

The mechanism of host restriction of the replication of infecting Friend mouse leukemia virus apparently involves interference possibly by a RNA, with the synthesis of competent proviral DNA. In other studies, the integrated proviral DNA of simian sarcoma virus in the cell chromosome was shown to be organized in nucleosomal structure analogous to host DNA.

Studies were initiated on cellular regulatory genes influencing endogenous virus gene expression. A number of representative genetic loci have been selected for study in congenic strains of mice. In addition, loci influencing xenotropic virus expression are being identified by genetic crosses between selected inbred mouse strains with different levels of endogenous virus expression. The induction of tumors by chemical carcinogens was shown to be genetically determined and related to the loci influencing virus expression. Several genes were shown to control expression of endogenous virus in chickens. In the chicken, present evidence suggests that elevated levels of virus expression may coincide with increased risk of spontaneous tumor development.

A number of studies have been in progress to correlate such factors as interpersonal and animal contacts, race, genetic tracts, previous virus infections and immunological status with the development of malignant diseases in humans. No evidence was obtained associating interpersonal or animal contacts with cancer in humans. On the other hand, HLA type has been associated with susceptibility to nasopharyngeal carcinoma, lymphocytic leukemia and prostatic cancer. Certain endocrine dyscrasias were linked with breast cancer and exposure to environmental chemical carcinogens and EBV infection with nasopharyngeal carcinoma. The specific immune reativity against antigens of certain cancers found to exist among close associates and relatives of some patients suggests that transmissible factors may be present. Recently, a type C virus, not yet completely characterized, was recovered from a human histiocytic lymphoma cell culture. This virus is related but not identical to the simian sarcoma virus.

Immunosuppressed organ transplant patients are at high risk of cancer. Based on previous experimentation on control by interferon of endogenous virus activation in immunosuppressed mice bearing tissue grafts, studies were initiated to determine the effectiveness of interferon in the control of virus diseases activated in organ transplant patients. This work has not progressed to a sufficient degree for evaluation.

Host immune responsiveness to virus associated or tumor cell associated antigens holds promise as a tool in diagnosis or prognosis for some cancers. Patient immune status to EBV related antigens correlates with the risk of primary Burkitt's lymphoma or relapse following therapy, and with the clinical status of nasopharyngeal carcinoma. Cell immune reactivity to EBV antigens also correlates with disease status in some cases. Lymphocyte cell surface markers may prove of value in characterizing certain childhood leukemias for prognosis and therapy. The use of fluorescein-tagged concanavalin A is being evaluated for diagnosis of Hodgkin's disease.

Cytoreductive therapy of a virally-induced neoplasm may leave the host susceptible to recurrence of a tumor. In both the AKR mouse lymphoma and the C3H mouse mammary tumor systems, evidence for protection against recurrence following tumor ablation or cytoreductive therapy was obtained in animals inoculated with neuraminidase-treated syngeneic and allogeneic tumor cells. These observations were applied in other investigations on human acute monocytic leukemia. A remarkable increase in the period of remission was obtained when chemo-immunotherapy, using neuraminidase-treated allogeneic myeloblasts, was applied.

The prophylactic efficacy of active and of passive immunization of mice against development of neoplasia has been examined. Some inhibitory efficacy was observed in cogenitally acquired lymphoma in susceptible mice that were previously passively immunized with viral antigens. Prior active and passive immunization also had a protective effect against chemically induced tumors. The presence of antibody to fetal antigens in multiparous hamsters provides resistance to SV40 induced oncogenesis. A new adoptive immunotherapy model is of interest. In this system, syngeneic or allogeneic macrophages that have been exposed to a tumor antigen are used to sensitize syngeneic lymphocytes *in vitro* rendering these cells capable of reacting *in vivo* in a specific Winn test.

Previous studies showed that cultured murine cells, when infected by a murine leukemia virus, were more sensitive to transformation by chemical carcinogens than were uninfected cells. Other observations showed that several classes of chemical carcinogens, other viruses, and irradiation could activate endogenous virus gene expression in cultured murine cells. During the past year, new projects were initiated to study the interaction between viruses and other environmental factors in relation to the neoplastic process.

Experimental results indicate that the Fl706 line of Fischer rat embryo fibroblasts in early passage is useful for screening chemical compounds for carcinogenic activity. The results of morphological transformations of these cells is being correlated with mutagenesis of *S. typhimurium*. Two mouse cell lines are being evaluated for studies on the role of type C virus expression as a cofactor in chemically induced carcinogenesis. Virus expression is activated in these lines by treatment with iododeoxyuridine and dexamethasone.

Studies *in vivo* were made using crosses of NZB x 129 mouse strains and NZB x SWR mouse strains. Present data obtained from these crosses indicates that a single autosomal dominant gene regulates endogenous xenotropic virus expression. The sensitivity of the mice to 3-methylcholanthrene induced fibrosarcomas was found to correlate with the level of xenotropic virus expression. These

animal systems will be used for further study of the interaction between the virus and 3MC and their causal role in tumorigenesis.

If viruses are cofactors in chemically induced carcinogenesis in murine animals, the suppression of virus gene expression or virus production might inhibit carcinogen induced cancers. Some studies were made using the F1706 line of Fischer rat embryo cells. Geldanomycin showed protective activity against transformation of cells by 3MCA. The protected cells did not grow to produce tumors when inoculated into newborn rats. Similarly, F1706 cells treated with anti-rat leukemia virus IgG 2 or 3 days prior to exposure to 4-nitroquinoline oxide did not exhibit a morphologic transformation and did not grow to produce tumors when inoculated into newborn rats. Studies are in progress to determine whether the cellular toxicity of the IgG is related to the inhibition of transformation.

b. SUMMARY REPORT

(1) BREAST CANCER VIRUS STUDIES SECTION

July 1, 1976 through September 30, 1977

The principal aim of the Breast Cancer Virus Studies Section is to achieve an understanding of the fundamental biology of mammary tumor pathogenesis and to determine whether a viral agent is etiologically involved in human mammary gland malignancies. The approach has been to identify a putative human cancer virus and determine the genetic, hormonal, immunological and environmental factors that govern its expression. This information will be useful in the development of improved diagnosis, treatment and possible prevention and control of breast cancer in humans. Fifteen research contracts administered by this section have been investigating the probable causal relationship of viruses to breast neoplasia.

Human Studies: At the present time, mammary gland cancer is known to be caused by a virus in only one animal species, the mouse. One of the major activities of the Breast Cancer Virus Studies Section this year has been to apply information and leads gained from the mouse mammary tumor virus (MMTV) system to determine whether an association exists between viruses and breast malignancies in humans and in animals other than mice.

The results of studies during this period suggest that viral-related activity may be present in human materials, but in relatively low amounts. Investigators at the Institute for Medical Research reported that certain high-density concentrates recovered from pools of human milk contain antigens which cross react with antisera to MMTV-glycoprotein-gp55; whereas, samples obtained from individual women gave negative results when tested by the same radioimmune assay. Along the same line, the University of California at Davis group found that the analysis of tissue extracts from individual breast cancer patients for inhibition of radioimmune precipitation by antisera to intact mouse mammary tumor virus (MMTV), gp52 and gp36 were negative. In other clinical projects, sera, breast duct and cyst fluids were screened for the presence of antigens related to MMTV and Mason Pfizer Monkey Virus (MPMV). Although these fluid samples examined in collaborative studies under the Howard University contract contained relatively negligible levels of reverse transcriptase activity, some fluids showed positive reactivity against MMTV and MPMV by the immunofluorescence assay. Dr. Bowen, at the University of Texas reported that some patients with breast cancer had serum antibodies to MMTV-producing mouse mammary tumor cells. In the majority of sera such reactions could only be removed by absorption with MMTV. Similarly, cells of some human breast tumor lines produced antigens which reacted with anti-MMTV sera.

Further information has been gathered on the cell-mediated immune response of breast cancer patients to a variety of tumor and viral related reagents under the New York Medical College contract. Patients classified as lymphoreticulo-endothelial (L-RE) positive, gave positive cellular responses to MMTV gp55 from RIII mouse milk and extracts of MCF-7 human breast cancer tissue culture cells.

In contrast, L-RE negative patients had lower in vitro reactions to the same materials. That is, increased cellular reactivity against viral and tumor related antigens seems to be correlated with the patient's L-RE characteristics.

Many aspects of the biology of breast cancer can best be approached with the development of human mammary epithelial cell lines. The University of California at Berkeley has been successful in developing two long-term continuous mammary cell lines, one normal and the other malignant, from the same breast of a mammary tumor patient. Such substrates should prove to be invaluable in studies on the etiology and control of human mammary cancer. Under the same project, preliminary data suggested that there may be specific ultrastructural markers that are more prevalent in malignant cells than in normal cells. Extension of these observations are underway.

The primary focus of the Michigan Cancer Foundation contractor during this period has been to enhance the production of the 734 B virus particle isolated from the MCF-7 human breast cancer cell line. Although the systematic manipulation of the culture milieu only resulted in a 2-4 fold increase in virus expression, it now appears that the main reason for the low yield may be attributed to the fact that MCF-7 cultures are pleomorphic mixtures of large (L) and small (S) cells, in which only the L cells are active in 734 B synthesis. Attempts will be made to isolate and stabilize clones of L and S MCF-7 cells for further characterization and virus induction.

Animal Studies: Most mammary tumor research has been devoted to the study of breast cancer in laboratory mice. Although this work has yielded much valuable information, a closer model to human cancer is needed. Two separate projects were set up this year to determine whether new animal model systems for breast cancer research could be established.

One of the projects at the Sloan-Kettering Institute for Cancer Research is the study of mammary tumors of cats. Intracisternal A particles were observed in some of the tumors examined to date and the same specimens have shown immunologic reactivity with antisera to MMTV viral antigens. The second project at Pfizer, Inc., places primary emphasis on cell culture and chemical induction techniques in attempts to activate virologic activity in mammary cancers of dogs. Thus far, only minimal viral-related information was detected and a few virus-like particles were observed in biopsy and tissue culture material derived from several dogs.

Since the Mason Pfizer Monkey Virus (MPMV) was implicated as possibly having an etiologic role in breast cancer, a third contract with Mason Research Institute involves the inoculation of MPMV into rhesus monkeys in efforts to determine whether the virus is oncogenic in its natural host. These studies necessitate long term holding of virus inoculated and uninoculated monkeys with continuous monitoring of MPMV p27 antigen levels and pertinent clinical endpoints. As yet, there has been no evidence of grossly palpable mammary tumors in any of the experimental groups; however, many of the neonatally infected animals are still too young to expect neoplastic changes to

be evident macroscopically. Other results indicate almost uniformly the presence of p27 antigens in infected monkeys as well as in certain hormonally treated animals inoculated with MPMV as adults. All uninoculated control animals have remained negative.

Virus Characterization Studies: Considerable progress has been made in demonstrating the in vitro chromatin transcription of mouse casein mRNA as a model system for gene function in mammary tissues. A hybridization probe for mouse casein mRNA was prepared and its complementary DNA copy was made. Investigators at Baylor College of Medicine are in the process of duplicating this effort with a hybridization probe synthesized from MMTV-RNA. When sufficient amounts of the probe become available, the contractor will determine whether it is possible to transcribe MMTV in vitro using chromatin as a template for synthesis.

Several lines of evidence have suggested that there may be more than one strain of mouse mammary tumor virus. Dr. Cardiff, after examining MMTV from RIII mouse milk and C3H mouse tissue culture, reported differences in the mobility and intensity of staining of viral polypeptides derived from the two sources. Investigators from the Institute for Medical Research found that gp55 isolated from RIII strain mice differed in molecular weight and tryptic peptide mapping from gp50 obtained from A strain mice. Quantitative amino acid and carbohydrate analyses of these and other polypeptides are in progress. At least two strains of mouse mammary tumor virus were identified by researchers at the Radiobiological Institute and the Netherlands Cancer Institute. Investigators at the Radiobiological Institute presented genetic evidence that one single dominant gene, designated Mtv-1, controls the release of MMTV-L (a low-oncogenic variant of MMTV) in C3Hf mice. Another single gene, Mtv-2, appears to be responsible for the release of endogenous MMTV-P, a virulent virus which causes hormone-dependent tumors in GR mice. C57BL mice apparently harbor a single epistatic gene, designated Imv, which can inhibit the release of MMTV-P. The presence of the MTV-1 and MTV-2 loci have been confirmed and extended at the Netherlands Cancer Institute, where investigators reported that the MTV-2 locus of the GR mouse strain consists of eight multiple MTV-DNA copies arranged in tandem. The MTV-1 locus, not allelic with Mtv-2, is located on the first linkage group. Several congenic mouse strains are being developed by these investigators for further genetic analysis.

Researchers at the University of California at Davis conducted studies to determine the location of MMTV polypeptides within the virion. In summary, gp52 and gp36 envelope proteins are probably located on the surface spikes of the virion, p14 and p28 in the internal core structure and p10 may be located somewhere between the external membrane and the core.

In MMTV infectivity studies, at the Institute for Medical Research, it was found that infected cat kidney and mink lung cells contain unintegrated MMTV DNA molecules as well as the integrated provirus. In additon, glucocorticoid treatment seems to stimulate MMTV RNA synthesis in these cells. During the year, this group also was successful in infecting an epitheloid cell line derived from a C57BL mouse non-lactating mammary gland which normally shows very low levels of MMTV RNA synthesis. After infection, additional MMTV

proviral DNA sequences were detected. The infected cells synthesized MMTV-RNA and produced budding B particles. In other experiments, a human breast tumor line also appears to have been infected with MMTV and is apparently synthesizing low levels of virus. Extension of these extremely important studies is underway.

In a series of experiments to study the effects of immunization of mice with MMTV polypeptides, investigators at the Institute for Medical Research found that C57BL mice immunized with gp55 without adjuvant and p28 with adjuvant had no effect when the mice were challenged with infective RIII virus. However, gp55 in Freund's complete adjuvant at some doses appeared to partially suppress viral replication in these mice. In other studies, the Radiobiological Institute reported that when BALB/c, C3Hf, GR and BALB/cfC3H were vaccinated with a protein vaccine of MMTV enriched for gp52, a 2-3 month delay of tumor onset was observed in BALB/cfC3H and GR mice. In short-term studies on the induction of transplantation resistance by MMTV protein vaccines, low doses of the vaccine in combination with adjuvant was more protective against tumors containing MMTV, than adjuvant alone. It was also found that higher doses of MMTV protein cause acceleration of tumor growth.

Mammary tumors of mice proved to be suitable models for the resolution of some of the complexities of host-tumor interactions. At the University of Miami, it was shown that MMTV-negative Balb/cCrql mice whose lymphocytes respond to MMTV antigen *in vitro* appeared to be more resistant to tumor growth than MMTV-positive mice. In contrast, lymphocytes from MMTV-positive Balb/cfC3H mice did not react or reacted only slightly to MMTV antigens until the mice received transplants of MMTV-positive, spontaneously arising, mammary tumors. The immunologic behavior of both sublines of mice with respect to mouse mammary tumor virus was distinctive and it may well be that positive cell-mediated immune reactions in mice toward MMTV affords a protective effect to the host.

BREAST CANCER VIRUS STUDIES SECTION

Dr. Clarice Gaylord, Head

BAYLOR COLLEGE OF MEDICINE (NO1-CP4-3385)

Title: Regulation of Gene Expression in Mouse Mammary Cancer

Contractor's Project Director: Dr. Susan H. Socher

Project Officer (NCI): Dr. Wade Parks

Objectives: Examine the regulation of gene expression in cells using murine mammary tumor virus (MMTV)-RNA and casein mRNA as specific markers of gene function in mouse mammary tissue. Analyze the expression of these individual genes in normal mammary tissues, prenoeplastic alveolar nodules and mammary tumors in both MMTV-positive and MMTV-negative lines of mice. Quantitate levels of MMTV and casein RNA sequences, using selective hybridization probes, in RNA transcripts synthesized in vitro from isolated chromatin templates and in RNA extracts isolated from these same tissues. Study mechanisms by which chromatin-associated molecules modulate transcription of these genes.

Major Findings: Purification and characterization of 15S mouse casein mRNA and its complementary DNA (cDNA) copy were carried out. The mRNA was labeled with radioactive iodine and used to determine if the cDNA generated represented a full copy. The full length cDNA probe was used to measure levels of casein mRNA in tissue extracts and in chromatin transcription products. Although casein mRNA is present during mammary gland development and hyperplastic alveolar nodules (HAN), no casein sequences were detected in hormone-independent tumors. An anticomplementary DNA probe for casein mRNA was generated for analyses of the fidelity of chromatin transcription.

Isolated viral RNA, avian myeloblastosis virus (AMV) reverse transcriptase and calf thymus DNA were used for synthesis and characterization of MMTV-cDNA. The specificity of the MMTV-cDNA was demonstrated by its lack of hybridization with murine leukemia virus (MuLV)-cDNA and its back hybridization value of 3×10^{-2} moles sec liter^{-1}. This sensitive and selective MMTV-cDNA hybridization probe is used for analysis of the levels of transcription of MMTV during mammary gland tumorigenesis.

The system used for the in vitro chromatin transcription studies reproduced casein mRNA synthetic patterns of the tissue of origin. Evidence obtained leads to the postulation that run off does not occur within the in vitro transcription system used. Studies to demonstrate chain initiation in vitro and hybridization analyses to determine possibilities for transcription of the MMTV gene in vitro using chromatin as a template for synthesis were initiated.

Significance to Biomedical Research and the Program of the Institute: Considerable evidence suggests that control of the inherited mouse mammary tumor virus genome occurs at the level of DNA transcription to RNA. These studies are designed to obtain an increased understanding of the regulatory mechanisms of

the effects of hormones in differentiation, and on the regulation of viral and non-viral genes involved in carcinogenesis.

Proposed Course: Present efforts focus on in vitro chromatin transcription of MMTV-RNA and the initiation of MMTV and casein RNA transcription in vitro.

Date Contract Initiated: June 26, 1974

Current Annual Level: $96,036

CALIFORNIA, UNIVERSITY OF, BERKELEY (NO1-CP5-3502)

Title: Human Mammary Tumor Biology: Development of Epithelial Cell Lines

Contractor's Project Director: Dr. Adeline J. Hackett

Project Officer (NCI): Dr. Robert Bassin

Objectives: Develop mammary epithelial cell lines from normal and malignant human breast tissues for studies on the etiology and control of human mammary cancer.

Major Findings: Transmission and scanning electron microscopy showed subtle morphologic differences between normal, atypical and malignant cells. The shape of the cells, the morphology, location and number of microvilli on the cell surface seem to correlate well with the degree of malignancy. There appears to be consistant changes in the nuclear, nucleolar, mitochondrial, cytoplasmic and surface structures of neoplastic cells as compared to normal cells.

Two human epithelial cell lines, derived from the same breast, were developed. One line, 578T, was derived from a carcinosarcoma and is aneuploid with distinctive chromosomes, produces casein and is estrogen protein negative. The other, 578Bst, established from normal tissue peripheral to the tumor, is diploid and of myoepithelial origin. This is the first report of companion cell lines, one malignant and one normal, established from the same breast.

Newly developed myoepithelial cell lines, cocultivated with existing and primary cultures of breast cells, are assayed for changes in secretory activity, expression of differentiated functions such as casein, viral production and antigenic expression.

Myoepithelial cells appear to produce a diffusible factor which helps to promote epithelial cell growth. Almost all of the primary epithelial cells thus far tested grow readily on myoepithelial cell monolayers. This cell line promises to aid the development of epithelial cell lines.

Significance to Biomedical Research and the Program of the Institute: The establishment of human breast cell lines will provide materials for expanding investigations on virus relationships to the etiology of human breast cancer.

Proposed Course: The main effort will focus on clinical studies and cell biology next year. Additionally, screening breast cells for viruses will begin using fluorescein-labeled antisera prepared against Mason-Pfizer monkey virus, mouse mammary tumor virus and various primate type C oncornaviruses.

Date Contract Initiated: September 1, 1974

Current Annual Level: $129,000

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP6-1013; successor to NO1-CP3-3253)

Title: In Vitro Cultivation of Human and Mouse Mammary Tumor Virus

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Robert Bassin

Objectives: To develop and apply information from studies on mouse mammary tumor systems to the possible isolation and identification of a human mammary tumor virus.

Major Findings: Analysis of 26 human breast tissue extracts showed no inhibition of radioimmune precipitation of intact iodinated mouse mammary tumor virus (^{125}I-MMTV) by anti-MMTV, anti-gp52 and anti-gp36 sera. Extraction of MMTV antigens from C3H mouse tumors by the procedure used to extract the human breast tissues showed that MMTV antigenicity was preserved and that these antigens were detectable by the RIA-MMTV assay.

Monospecific antisera against MMTV polypeptides were generated and characterized. Radioimmunoassay for mouse serum IgM and IgA antibodies against MMTV and for individual MMTV polypeptides is under development.

Slab gel electrophoresis of intact, bald (protease-treated MMTV which lacks spikes but retains envelope and internal core structure) and core particles of MMTV established that 1) the envelope proteins gp52 and gp36 are present in the intact virions, but not the bald and core components; 2) p10, p14 and p28 polypeptides are present in the intact and the bald particles; 3) p10 is absent from the virus core while p14 and p28 are present. It is postulated that p10 is located between the external membrane and the core.

MMTV from two sources, milk and tissue culture, were examined for any inherent polypeptide differences by SDS-PAGE in 10-20% gradient slab gels, followed by Coomassie blue and PAS staining. Four to eight bands were resolved in the gp36 region; their mobility and intensity varied according to virus source. Marked differences were noted between virus from milk as compared to primary tumor cell culture virus. SDS gradient slab gels, in the presence of urea, accentuated differences in the gp52 region.

Results from analysis of fractions from detergent-treated MMTV suggest that RNA-directed DNA polymerases (RDDP) and terminal deoxynucleotidyl transferase (TdT) enzymes of MMTV may exist as a functional complex and further characterization is underway.

A representative MMTV cDNA probe from pools of DNA and RNA from hyperplastic outgrowth lines is being developed and characterized for hybridization experiments with the DNA and RNA extracted from various tissues. Electron microscopy and immunoperoxidase stain studies also are in progress.

MMTV production is being maintained by dome cultures initiated from mammary tumors of BALB/c mice foster nursed on C3H mice (C+), the MM3MT cell line and the GR cell line. Primary dome cultures of C+ mammary tumors have produced uncharacteristically low levels of MMTV (<10µg MMTV/T75/24 hrs.). However, the two most recent primary cultures have produced an average of 12 µg MMTV/T75/24 hrs. The GR3A cell line now at passage 6 has been a consistent MMTV producer at 15 µg MMTV/T75/24 hrs. without evidence of type C gs antigen.

Significance to Biomedical Research and the Program of the Institute: The acquisition of knowledge and technology on the in vitro cultivation of mammary gland tissue and the production and structure of MMTV is essential to an intensive program of investigation on virally-induced breast cancer in experimental systems and for application in virological studies on human breast cancer.

Proposed Course: The activities described will be continued at the current contract level.

Date Contract Initiated: February 1, 1972

Current Annual Level: $193,300

HOWARD UNIVERSITY (NO1-CP4-3287)

Title: Correlation of Molecular Virology Studies to Diagnosis of Breast Cancer

Contractor's Project Director: Dr. William Funderburk

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To determine whether there is a correlation between the presence of viral markers in spontaneous human nipple discharges, breast duct fluids, or cyst fluids and clinical stage of breast disease. Evaluation is made by comparison of virological and immunological results with palpation, xerography, pap smears of breast fluids by conventional and millipore techniques, and contrast mammography on the fluid donors.

Major Findings: During the past year, 38 patients were entered into the study for correlation of molecular virological observation on fluid specimens with clinical diagnosis of breast disease. Patients with nipple discharges or aspirated cysts were studied with pap smears, contrast mammography and xerography. Twenty-four duct secretions and 13 cyst fluids were collected and tested for the presence of reverse transcriptase activity. Because of relatively low enzyme activity in these fluids, studies were made to detect reactivity to Mason-Pfizer monkey virus (MPMV) and mouse mammary tumor virus (MMTV) using the fixed immunofluorescence assay. Of 53 cyst fluid samples tested, 5 were positive for MPMV, one was positive for MMTV and none were positive for both viruses.

Seven out of 90 duct secretion samples tested were positive for both MPMV and MMTV activity; whereas, only one and two duct secretions were positive for MMTV and MPVM, respectively. Sera from reactive patients in the latter group yielded a 40% positive response to both viruses.

Virological activity could not be shown to correlate with clinical course of breast disease.

Significance to Biomedical Research and the Program of the Institute: These studies were conducted to obtain fundamental information that could contribute directly to the NCI objectives of better diagnostic methodology for breast cancer. Further, these breast fluids were a likely source of a possible human breast cancer virus.

Proposed Course: This contract terminated on February 28, 1977.

Date Contract Initiated: March 1, 1974

Current Annual Level: $10,995

INSTITUTE FOR MEDICAL RESEARCH (NO1-CP5-3561)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: (1) To characterize the mammary tumor system of mice as a model for human breast cancer. (2) To resolve whether there is any relationship between murine mammary tumor virus (MMTV) and human cancer. (3) To determine the significance of virus-like particles resembling oncogenic RNA viruses present in some human milks and breast tumors.

Major Findings: Whole human milks were pooled without regard to age, history of breast cancer, and parity, skimmed by centrifugation and fractionated by zonal centrifugation. Of the fractions that were recovered from the high-density pool, five contained antigens similar to antigenic determinants in MMTV glycoprotein gp55. Pellets obtained by centrifugation from individual samples from 42 women were negative by radioimmunoassay for the presence of antigens related to MMTV gp55 indicating either a very low concentration or infrequent occurrence of gp55.

Characterization of RNA-directed DNA polymerase (RDDP) activity from a particulate fraction of pooled human milk revealed a polymerase with activity on poly rC-oligo dG template primer eluting at a salt concentration of approximately 0.15 M NaCl. Preliminary results indicate that the RDDP of human milk has some common properties (template and cation requirements) and some properties (molecular weight, phosphocellulose elution) that are different from MMTV-RDDP.

A comparative study of immunologically related purified structural proteins and glycoproteins from MMTV's derived from various mouse strains were initiated. Gp55 isolated from RIII strain mice and gp50 from A strain mice, which are immunologically related and PAS-positive proteins, differed by molecular weight on the basis of SDS-PAGE and Sephadex G-100 gel filtration. Preliminary tryptic peptide mapping of these two proteins disclosed differences, but it is not known whether these differences are due to amino acid sequence differences or differences in carbohydrate content. Clarification awaits completion of quantitative amino acid and carbohydrate analyses.

Since formalinized virus was shown to protect C57BL strain mice against subsequent RIII mouse virus challenge, a series of experiments was begun to study the effects of immunization with MMTV components. Proteins gp55 and p28 were purified from RIII strain mouse virus and gp50 from A strain mouse virus. C57BL mice immunized with RIII virus gp55 or p28 and challenged with RIII MMTV showed no significant resistance to infection. Tumor incidence is not yet available.

MMTV was shown to infect cultured cells from animals other than mice. Even in newly infected non-murine cells, MMTV RNA synthesis was stimulated by glucocorticoids indicating that either MMTV proviral DNA integrates adjacent to a host cell glucocorticoid receptor binding site or that MMTV proviral DNA itself may contain a site for binding of a glucocorticoid-receptor complex.

Cat and mink cells infected in vitro contained unintegrated MMTV DNA molecules in both circular and linear forms. A C57BL mouse mammary epithelial cell line was infected with RIII virus. Logarithmic dilution of the inoculum resulted in a logarithmic reduction in the average number of MMTV RNA molecules detected per cell when tested four weeks after infection. A cell line derived from a human breast carcinoma was infected with MMTV. Although these cells synthesize extremely low levels of MMTV RNA (0.8 copies/cell), the proviral DNA is present at about 1 to 2 copies/cell.

Significance to Biomedical Research and the Program of the Institute: Antigenic determinants related to MMTV antigens have been detected in some human breast cancer specimens. Determination of the nature of this relatedness is important to the question of viral association with the human disease. The infectivity of MMTV for cells of human and non-murine animals species raises the possibility of a distant common viral progenitor perpetuated by vertical transmission within different species but still retaining some antigenic similarities. Viral immunization of mice to determine prospects for disease control is consistent with the objectives of the program

Proposed Course: This contract terminated on April 30, 1977.

Date Contract Initiated: June 28, 1968

Current Annual Level: $341,000

MASON RESEARCH INSTITUTE (NO1-CP6-1052; successor to NO1-CP3-3358)

Title: Studies on Mammary Tumors in Rhesus Monkeys Infected with Mason-Pfizer Monkey Virus

Contractor's Project Director: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To determine whether the Mason-Pfizer Mammary Virus (MPMV) is oncogenic in monkeys.

Major Findings: Breast palpations for tumor nodules on the MPMV-inoculated primate colony detected no breast lesions. All animals are maintained in isolation from other primates; biohazard precautions are applied. Ninety-four subhuman primates were screened by radioimmunoassay (RIA) for expression of MPMV p27. Among the various primate species inoculated with MPMV as neonates, 92% (34/37) were positive, and of those that were inoculated as juveniles or adults, 61% (20/23) were antigenemic. All uninoculated animals were negative (0/22).

MPMV p27 antigenemia was demonstrated in 2 juvenile rhesus that were not inoculated but were born to inoculated mothers. Both had been suckled by their mothers and one mother received an MPMV inoculation during the post-partum lactation period.

Rhesus females, inoculated neonatally with MPMV and positive for p27, showed fluctuations in serum antigen levels in response to exogenous estrogen treatment. Animals with initially negative sera remained negative despite estrogen treatment.

In a comparative study involving 25 mature rhesus females, MPMV p27 antigenemia was demonstrated in 3 of 9 animals that received an intramammary ductal inoculation following 17β-estradiol therapy, and in 7 of 9 that received an intramammary alveolar inoculation following combined 17β-estradiol and progesterone therapy. Eight uninoculated control animals treated similarly with steroids remained negative.

Serial serum samples from two rhesus inoculated intravenously with MPMV while implanted with estradiol pellets, remained negative for MPMV antigen despite chronic immunosuppression induced by total body X-irradiation. Significantly, the mammary glands from both monkeys were found to be positive by RNA-DNA hybridization test.

Urine samples from 7 monkeys were tested for MPMV p27 by radioimmunoassay. The presence of MPMV p27 was demonstrated in the urine of the original I-9 monkey and 3 neonatally inoculated rhesus with antigenemia, wherease the urine of 3 uninoculated non-antigenemic rhesus was negative.

Serum samples of 6 persons who have been associated with MPMV inoculated animals for 1-4 years were negative for MPMV antigenemia.

Significance to Biomedical Research and the Program of the Institute: MPMV was recovered from a primate mammary cancer, it possesses characteristics common to known oncogenic viruses, and cross-reactions have been observed between the viral antigens and antigens present in human breast cancer specimens. Investigation for possible oncogenic properties in a primate host is essential.

Proposed Course: Continuation of the studies described and collection of data necessary for decisions concerning continued long-term holding and observation of these animals.

Date Contract Initiated: June 9, 1970

Current Annual Level: $86,988

MIAMI, UNIVERSITY OF (NO1-CP5-3532)

Title: Immunologic Studies on Animal Breast Carcinoma

Contractor's Project Director: Dr. Michael M. Sigel

Project Officer (NCI): Dr. Meera Paranjpe

Objectives: (1) To determine, define and integrate the relevant factors in host defense against mammary tumors; (2) to analyze cellular immunity in relation to clinical state; and (3) to compare lymphocyte responses to mammary tumor virus (MMTV)-associated, MMTV-induced and non-MMTV tumor-specific antigens in mice with breast cancer.

Major Findings: Lymphocytes from Balb/cCrgl mice, free of infectious MMTV, were shown to respond to RIII mouse milk and to purified MMTV from the same source. The specificity of this reaction was proven by neutralization tests. Using MMTV purified from a cell culture derived from the C3H mouse strain, it was found that lymphocyte reactivities to MMTV from the two different sources were not identical.

Immunofluorescence assays were used to localize the antigen which may be responsible for the sensitivity of Balb/c lymphocytes to MMTV. The studies included lymphoid cells from MMTV-negative Balb/c mice and MMTV-positive Balb/c mice foster nursed on C3H mice. Thymocytes from both were uniformly negative in all tests. HIgh percentages of splenocytes from the foster nursed Balb/c mice were postive by immunofluorescence. Lower levels of fluorescing cells were observed in Balb/cCrgl mouse splenocytes. Splenocytes from Balb/cCrgl mice bearing D1-DMBA-3 tumors gave, as a rule, higher percentages than normal mice.

Studies were undertaken to search for suppressor factors in the spleens of mice bearing large mammary tumors. In lymphocyte transformation assays a suppressive activity was detected against the mitogenic response induced by phytohemagglutinin in normal Balb/c mice. Addition of splenocytes in increasing concentrations from animals bearing large D1-DMBA-3 tumors resulted in severely depressed levels of 3H-thymidine incorporation, which could not be accounted for by dilution factors only.

Migration inhibition and lymphocyte transformation assays were performed to elucidate the relationship between temporal aspects (days after implantation, days after surgery) and the responses of the animals to viral and tumor antigens. Positive responses were obtained with MMTV antigens in both normal and tumor-bearing mice in all combinations. Responses to tumor extracts appeared 7 days in animals implanted with tumors. There were no significant differences among the results with the various protocols. The major condition which seemed to determine a change in the response to tumor antigens and mitogens was the recurrence of the tumor.

The responses to tumor antigens and MMTV in lymphocytes of Balb/cCrgl mice bearing mammary tumors do not seem to be governed by the same factors. Therefore, it was deemed necessary to see which cell type is involved in each of these responses. Preliminary results indicate that responses to viral antigens might be due to the B cell fraction.

A new kind of cytotoxic reaction SINEC (specifically induced nonspecifically expressed cytotoxicity) was described. Preliminary studies on the mechanism of action indicate that a lymphotoxin apparently is responsible, at least in part, for the damage to the target cells. The SINEC reactions were seen only in tumor-bearing mice, and there appeared to be a correlation between degree of SINEC and tumor size. This is in sharp contrast with the results obtained with the migration inhibition and lymphocyte transformation assays, where increases in tumor size cause profound depressions in the lymphocyte responses. This important observation will be the basis of future studies.

Significance to Biomedical Research and the Program of the Institute: The murine mammary tumor viruses (MMTV) are etiologically related to breast cancer in mice and there is some evidence that similar viruses may be present in humans. Because of the laboratory and clinical similarities between breast cancer in mice and in women, the present work is designed to obtain a clearer understanding of the immunological reactivity of the host to an array of MMTV and tumor-related antigens. The approach is to identify various antigens and immunologic as well as non-immunologic factors governing the relationship and interactions of host, virus and tumor. This project will provide information relevant to both etiology and control of breast cancer in humans.

Proposed Course: This study will continue to assess the immunologic status and response in mice with different virological and immunological backgrounds and to correlate these responses to various clinical stages of mammary gland neoplasia.

Date Contract Initiated: March 15, 1974

Current Annual Level: $142,464

MICHIGAN CANCER FOUNDATION (NO1-CP3-3347)

Title: Studies of High Risk Breast Cancer Families

Contractor's Project Director: Dr. Marvin A. Rich

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: (1) To characterize virus particles (734B) produced by the human breast tumor cell line MCF-7; (2) to identify and characterize human populations at high risk for breast cancer, seeking to provide early detection and control of breast cancer; and (3) to study model systems to elucidate the role of viruses in the genesis of human breast cancer.

Major Findings: The MCF-7 cell population is comprised of multiple phenotypes. Even in late passages (150 and above), two phenotypes could be distinguished readily by morphologic and histochemical criteria. The first type of cell (L-cell), is large and polygonal and is interconnected only loosely with adjacent cells. It contains abundant cytoplasm poorly stained by methylene blue. The second type of cell (S-cell), one-third to one-half the size of L-cells, is tightly interconnected with adjacent cells and displays a much larger nuclear/cytoplasmic ratio. X-cell cytoplasm is much more basophilic than L-cell cytoplasm, as shown by staining with methylene blue. In monolayer culture of late passage MCF-7, 80-90% of the cells are S-cells; only 10-20% are L-cells. The L-cells generally exist as small islands amidst the S-cells.

To determine the level of control over 734B particle replication of MCF-7 cell populations, 734B-specific cDNA was prepared from endogenous 734B reverse transcriptase (RT) reactions and used in hybridizations with the DNAs of producer and non-producer cell populations. The results showed that both populations shared equally in 734B DNA content and, therefore, both populations were potentially capable of generating the particle. Parallel studies showed that non-producer MCF-7 cell populations contained less 734B RNA per cell than producer populations, suggesting that MCF-7 cells control 734B synthesis either by regulating 734B RNA transcription or RNA catabolism.

To attempt improvement of 734B release from MCF-7 cells, hormones were added to the cells. This was based on the earlier observation that progesterone was capable of activating both 734B release and MMTV related antigen synthesis in MCF-7 cells. Steroid hormones, represented by a receptor in MCF-7 cells (e.g. estrogen, progesterone, androgens, glucocorticoids), were used most extensively in attempts to activate 734B. None of the many hormones or hormone combinations tested resulted in a substantial increase in RNA-directed DNA synthetic activity without concomitant increase in contaminating polymerases. Polypeptide hormones, including insulin, thyroxin, somatotropin and prolactin, added in concentrations from 1 to 10μg/ml, did not augment 734B release.

Non-hormone compounds, including DMSO, IUdR, BUdR and cycloheximide, all activators of RNA tumor viruses in experimental systems, were ineffective for 734B activation in MCF-7 cells.

In addition to immunologically cross-reactive antigens, MMTV-related RNA was also detected in MCF-7 cells by hybridization with MMTV cDNA. Under the same conditions, MMTV-related RNA was not detected in homogenous cell populations of two non-breast malignancies in culture. MuLV (Rauscher) cDNA did not hybridize with MCF-7 RNA, suggesting not only the absence of adventitious murine type C virus contamination of MCF-7 cells, but also that MCF-7 cell RNA does not randomly complex with viral cDNA at the RNA concentrations used. The stringency of the hybridization conditions suggests that the hybridizable MCF-7 RNA has extensive sequence homology with the MuMTV cDNA probe.

Mason-Pfizer monkey virus related RNA has not been detected in MCF-7 cells using MPMV cDNA representing a minimum of 60% of the MPMV genomic RNA. The major internal p27 protein of MPMV also was not detected by immunofluorescence assays or by more sensitive radioimmunoassays. Cocultivation of MCF-7 cells with MPMV-susceptible NC37 cells has not shown MPMV RNA synthesis or virus replication in the cultures.

MCF-7 cells transplanted into nude mice produced only a 3.3% incidence of tumors. The histopathology of the tumors was that of an infiltrating ductal carcinoma, the primary tumor from which MCF-7 cells were obtained.

The growth of MCF-7 cells in culture is stimulated by estradiol and inhibited by the anti-estrogen, Tamoxifen and dexamethasone. Growth inhibition by Tamoxifen is overcome by estradiol.

Studies were initiated to determine which lymphocyte dependent immunological activities regulate the host's cellular immune response to MMTV-induced mammary tumor cells. Experiments are being designed to determine the nature and efficacy of the response to MMTV antigens in the MCF experimental model system for ultimate application to the human disease.

Significance to Biomedical Research and the Program of the Institute: These studies concern the isolation and characterization of a possible human breast cancer virus.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: June 20, 1971

Current Annual Level: $347,356

NETHERLANDS CANCER INSTITUTE (NO1-CP3-3368)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Directors: Dr. L. M. Boot
Dr. J. Hilgers

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To study the immunogenetics of mouse mammary tumors and mouse mammary tumor virus (MMTV) transmission, as well as that of leukemia and murine genes for transmission, MMTV replication, histocompatibility, and immune response.

Major Findings: It has been shown that the Mtv-2 genetic locus of the GR mouse strain, which is responsible for high levels of MMTV in the milk and for hormone-induced early mammary tumors, consists of about eight MMTV-DNA copies arranged in tandem. Such studies were possible using the "proviral-congenic" mouse strains developed on the contract. The Mtv-1 locus, not allelic with Mtv-2 and located on the first linkage group, was found to be responsible for low levels of MMTV antigen in the milk of C3Hf mice and for mammary tumorigenesis at about one year of age in the Balb/cx (Balb/cx C3Hf) backcross. The genetic resistance of the H-2 haplotype to exogenous MMTV (C3H-MMTV) induced mammary tumorigenesis was shown not only on the B10 background, but also on the C3H and Balb/c backgrounds, using H-2 congenic mouse strains. The H-2 haplotype does not interfere with mammary tumorigenesis by the endogenous C3Hf-MMTV (Mtv-1).

From F_1 hybrid studies with the high leukemia incidence strain, AKR, it was clear that the Fv-1^b allele can have a delaying effect on leukemogenesis, especially on the Balb/c and A backgrounds, but in Fv-1^{nn} hybrids leukemogenesis can also be delayed considerably in the presence of the high amounts of MuLV. The development of congenic strains for Akv genes on STS background is in its final stage.

All thymic leukemias (GRSL) occurring spontaneously in the GR strain express the MLr antigen related to MMTV on the cell surface. the MLr antigen is easily modulated (antibody-induced) from the GRSL surface in vitro and evidence has been obtained that modulation in vivo on ascites cells also occurs. The MLr-enriched membraneous vesicles isolated from the ascites fluid show a relatively high plasma membrane rigidity correlated with a high cholesterol/sphingomyelin content as compared to plasma membranes.

Significance to Biomedical Research and the Program of the Institute: Study of genetic factors controlling host susceptibility and capacity for internal control of oncogenic virus expression and infectivity is important to the program of this Institute. The results obtained thus far begin to delineate a variety of factors, in addition to the presence of an oncogenic virus, which contribute to or modify the pathogenesis of mammary cancer or leukemia. The findings described contribute to the investigations of possible etiological agents of human cancer as well as to greater understanding of the fundamental biology of cancer.

Proposed Course: The contractor proposes to continue studies concerning host genes involved in MMTV and MuLV expression in relation to mammary tumorigenesis and leukemogenesis and studies concerning viral antigen expression at the cell surface level and the immune response directed towards these antigens.

Date Contract Initiated: June 28, 1972

Current Annual Level: $90,000

NEW YORK MEDICAL COLLEGE (NO1-CP3-3398)

Title: Immunologic Measurements as a Guide to the Behavior and Viral Etiology of Breast Cancer

Contractor's Project Director: Dr. Maurice M. Black

Project Officer (NCI): Dr. Robert Callahan

Objectives: (1) To identify tumor- and virus-specific antigens in human breast cancer tissues; (2) to correlate measurements of patient immunologic reactivity with clinicopathologic characteristics of the tumor tissue and the host; (3) to search for evidence of the role of a mammary tumor virus in human mammary carcinogenesis by immunologic and biochemical procedures using mouse mammary tumor virus (MMTV) as a probe (in collaborative studies).

Major Findings: Studies of cross reactivity of breast cancer patients' leukocytes against RIII mouse virus-gp55 and autologous and homologous breast cancer tissues suggest that some breast cancer tissues contain both gp55-like antigen(s) and gp55-unrelated antigen(s), some contain either type alone and some seem devoid of both types. The MCF-7 human breast cancer cell line obtained from the Michigan Cancer Foundation provides a prototype of breast cancers with predominantly gp55-like antigenicity. Of 27 leukocyte preparations which were responsive to gp55, 59% cross reacted simultaneously with extracts of MCF-7. In contrast, of 54 leukocyte preparations which were not responsive to gp55 only 6% were responsive to MCF-7 extracts.

The relationship between in vitro leukocyte responsiveness to RIII-MMTV preparations and in vitro and in vivo responses to autologous breast cancer was examined. Among the L-RE positive breast cancer patients, in vitro leukocyte responsiveness to RIII-MMTV was associated with a high proportion of cross reactivity to autologous breast cancer tissue both in vitro and in vivo, viz. 86% and 72% respectively. These values are significantly greater than analogous values for L-RE negative cases whose leukocytes were responsive to RIII-MMTV but reacted with autologous breast cancer tissue in only 23% of leukocyte migration inhibition or skin window tests. These values were duplicated in similar studies with L-RE negative cases. Rabbit anti-gp55 antiserum blocked the in vitro response of breast cancer patients' leukocytes against gp55 and breast cancer tissues.

The specificity of the cell-mediated hypersensitivity against gp55 was also evidenced by the findings that gp55-positive leukocytes cross reacted in only 4 (21%) of 19 tests against gp50 derived from A-strain mouse. Since RIII virus-gp55 and A virus-gp50 possess cross reactive antigenicity when tested with anti-RIII virus-gp55 antisera, it appears that antigenic determinants responsible for cell-mediated immunity are different from antigenic determinants with which anti-gp55 antibodies react.

Significance to Biomedical Research and the Program of the Institute: Since the findings of this effort strongly suggest that the in vivo and in vitro cellular hypersensitivity responses of breast cancer patients provide biologically significant indices of maximal tumor-host interactions in the initial

stages of disease, the techniques used by the contractor may be useful tools in the possible early detection, diagnosis and effective treatment of breast cancer in women.

Proposed Course: During the next year, the contractor plans to expand studies on the correlations between protein characteristics of individual breast cancer tissues, immunogenic characteristics and MMTV proteins.

Date Contract Initiated: June 26, 1972

Current Annual Level: $175,780

PFIZER, INC. (NO1-CP6-1054)

Title: Studies of Viral Involvement in Canine Mammary Carcinoma

Contractor's Project Director: Dr. Mumtaz Ahmed

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To obtain evidence concerning the association of viruses with mammary neoplasias in dogs of various breeds.

Major Findings: The majority of tumors studied were papillary or tubular adenocarcinomas of the complex types. Both myoepithelial and glandular differentiation were apparent in mixed tumors. Of the five pairs of mammary glands in the dog, the inguinal glands were most frequently involved, next the abdominal pair, and less frequently the middle, thoracic and axillary glands.

Twenty-seven tissue specimens (normal, benign and malignant) from 13 dogs of different breeds, were established in culture using collagenase dispersion and tantalum screen explant culture techniques and four different growth media, enriched with various hormones and growth promoters (insulin, hydrocortisone, dexamethasone, prolactin, estrogen, progesterone). Most primary cultures, reflecting composition of tumors, contained mixed cell populations. Reverse transcriptase assay (using Mg^{++} or Mn^{++}), XC and KC tests, and EM examinations were made regularly on all dog cultures. A small amount of reverse transcriptase activity was observed in the supernatant concentrates (500 to 1000X) of some primary dog cultures and of some cocultivated and activated cultures. In most instances, the polymerase activity did not show a preference to Mn^{++} or Mg^{++}, and reproducibility was poor, XC or KC cells failed to produce syncytia when exposed to dog cultures. Although electron microscopic investigations have not shown typical budding retraviruses in dog cultures, a few virus-like particles were observed in biopsy and tissue culture materials derived from several dogs.

To determine whether malignant cells from dogs harbor ecotropic or xenotropic retraviruses, cell lines of homologous and heterologous species were routinely cocultivated with the dog mammary cells. In addition to the inclusion of various types of hormones in the growth medium, separate experiments were

carried out where dog cell monolayers were exposed to known inducers of type C and B viruses, e.g., 5-iododeoxyuridine, dexamethasone and streptozotocin.

Significance to Biomedical Research and the Program of the Institute: At present, the study of virus-induced mammary carcinoma is restricted to the mouse. Breast cancer is one of the most prevalent types of human cancer. Studies suggest that a virus may be associated with the disease in humans. Breast cancer also is among the most common neoplasms of dogs and resembles the human cancer in morphology, clinical behavior and incidence. Study of this malignancy in canines permits application of present knowledge to a systematic approach to elucidation of viral relationship to this carcinoma beyond the restriction to the murine system and increases prospects for increased understanding of viral roles in the human disease.

Proposed Course: To continue to examine mammary tumors, pleural effusions and milk secretions from several breeds of dogs for viral related information. Dog tissues established in culture will be cocultivated with cell lines of homologous and heterologous species and treated with chemicals in attempts to activate a putative viral genome.

Date Contract Initiated: June 25, 1976

Current Annual Level: $150,000

RADIOBIOLOGICAL INSTITUTE (NO1-CP4-3328)

Title: The Study of the Release of RNA Tumor Viruses and Its Genetic Control

Contractor's Project Director: Dr. Peter Bentvelzen

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To carry out studies in vivo and in vitro to elucidate the role of hereditary factors controlling the expression of genetically transmitted mouse mammary tumor viruses (MMTV) of mice. The importance of such genes in spontaneous mammary carcinogenesis will be evaluated in relationship to environmental agents such as chemical carcinogens and ionizing radiation. Some studies will also include the role of hormones in the control of these endogenous viruses.

Major Findings: By means of a sensitive immunoassay it was found that a single dominant gene in the C3Hf mouse strain controls the release into the milk of the low-oncogenic variant of murine mammary tumor virus (MMTV-L). The C57BL strain harbors a single epistatic gene, which can inhibit the release of MMTV-L. Although the gene had no effect upon the production of the virulent MMTV-P in the GR mouse strain, it appeared to impede the release of lesser oncogenic MMTV variants. MMTV-antigens were found in several organs of mice including some low mammary cancer mouse strains, which normally do not shed MMTV into their milk. For instance, in the CBA/Rij line, which does not release an MMTV into its milk and which has a low mammary tumor incidence, many organs were positive, including bone marrow, lung, kidney, salivary gland

and prostate. At one year of age the amount of antigen is increased in most organs, but decreases at two years. The amount of viral antigens in C57BL organs was usually insignificant. In the Balb/c strain several organs were positive at a young age. By plant lectin affinity chromatography followed by rechromatography on phosphocellulose columns or by gel filtration on Sephadex G75, gp52, gp36, p28 and p12 MMTV proteins were purified. In addition to free gp52 in the virion, there appears to be a complex of gp52 with several gp36 molecules.

The virus proteins gp52, gp36 and p28 produce single precipitation lines in the micro-Ouchterlony test. P12 did not give a line, but in the Sepharose bead immunofluorescence assay it proved to be quite reactive with an antiserum to MMTV-S.

Vaccination with a single dose of a MMTV protein fraction enriched for the major glycoprotein gp52 resulted in a slight but significant delay in spontaneous mammary tumor development. In short-term studies on the induction of transplantation resistance by MMTV protein vaccines, it was shown that low doses of the vaccine in combination with an adjuvant had a greater protective effect against occurrence of tumors containing MMTV than the adjuvants alone. High doses of MMTV protein caused acceleration of tumor growth. Studies, in vitro using the leukocyte adherance inhibition assay (LAI) indicated this acceleration was associated with a poor cellular reactivity to the virus, and the presence of specific blocking factors in the serum.

Significance to Biomedical Research and the Program of the Institute: The expression of MMTV in non-mammary gland tissue and the effect of cocarcinogens on the expression of MMTV is important to understanding the pathogenesis of virally-induced breast cancer and to prospects for the prevention or control of this disease.

Proposed Course: To continue the activities described at the current level.

Date Contract Initiated: April 26, 1974

Current Annual Level: $162,562

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP6-1053)

Title: Studies to Determine a Viral Involvement in Feline Mammary Carcinoma

Contractor's Project Director: Dr. Nurul H. Sarkar

Project Officer (NCI): Dr. John Dahlberg

Objective: To employ techniques of virus isolation, cell culture, immunology and biochemistry to elucidate a possible viral etiology in feline mammary carconoma.

Major Findings: During this first contract period, 43 feline mammary tumors were obtained. Electron microscopic, immunologic, biochemical and tissue

culture techniques were used to search for a putative feline mammary tumor virus in these tumors. Intracisternal type A particles were observed in 5 of the 29 tumors examined to date. Immunological studies using polyvalent anti-mouse mammary tumor virus (MMTV) serum detected reactive antigens in 5 of the feline tumors. A monospecific serum to MMTV gp34 reacted with a feline mammary tumor containing a large number of intracisternal type A particles, but the specificity of this reaction remains to be evaluated. Two feline mammary tumor cell cultures were established. One with epithelial morphology was characterized. Cells from this culture produced a tumor when injected into nude mice. The culture fluids had a low level of RNA-directed DNA polymerase (RDDP) activity. Biochemical studies showed that 3 feline mammary tumors contained RDDP.

Significance to Biomedical Research and the Program of the Institute: Currently, study of virus-induction of mammary carcinoma has been confined to mouse systems. Evidence suggests a virus may be associated with human breast cancer. Studies to extend knowledge of viruses in relation to this disease to other animal species than the mouse will yield greater insight into factors which bear upon the human disease.

Proposed Course: Cocultivation studies will be initiated to attempt to isolate and characterize virus from feline mammary tumor cells.

Date Contract Initiated: June 27, 1976

Current Annual Level: $150,000

TEL-AVIV UNIVERSITY (NO1-CP5-3517)

Title: Vaccination of Mice with Cell-Free Antigens Derived from MMTV and A Particles

Contractor's Project Director: Dr. Asher Frensdorff

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To test whether it is possible to immunize mice against mouse mammary tumor virus (MMTV) without exposing them, in the process, to structurally intact virus. To investigate whether immunization of mice with individual, purified structural proteins from MMTV can decrease or abolish their susceptibility to spontaneous as well as virus-induced mammary tumors. To isolate and purify the structural proteins under the mildest possible conditions to ensure conservation of the native conformation of the proteins. To immunize in such a way as to (a) increase as much as possible the immunogenicity of the proteins of which only very small doses are to be infected and (b) stimulate a cell-mediated, rather than humoral, immune response.

Major Findings: A goat antiserum was produced which is monospecific to gp52 by immunodiffusion and by radioimmunoprecipitation analysis and which does not react with antigens of murine leukemia virus (MuLV). A procedure was developed to isolate gp52 from disrupted MMTV at close to maximal yields; by affinity

and prostate. At one year of age the amount of antigen is increased in most organs, but decreases at two years. The amount of viral antigens in C57BL organs was usually insignificant. In the Balb/c strain several organs were positive at a young age. By plant lectin affinity chromatography followed by rechromatography on phosphocellulose columns or by gel filtration on Sephadex G75, gp52, gp36, p28 and p12 MMTV proteins were purified. In addition to free gp52 in the virion, there appears to be a complex of gp52 with several gp36 molecules.

The virus proteins gp52, gp36 and p28 produce single precipitation lines in the micro-Ouchterlony test. P12 did not give a line, but in the Sepharose bead immunofluorescence assay it proved to be quite reactive with an antiserum to MMTV-S.

Vaccination with a single dose of a MMTV protein fraction enriched for the major glycoprotein gp52 resulted in a slight but significant delay in spontaneous mammary tumor development. In short-term studies on the induction of transplantation resistance by MMTV protein vaccines, it was shown that low doses of the vaccine in combination with an adjuvant had a greater protective effect against occurrence of tumors containing MMTV than the adjuvants alone. High doses of MMTV protein caused acceleration of tumor growth. Studies, in vitro using the leukocyte adherance inhibition assay (LAI) indicated this acceleration was associated with a poor cellular reactivity to the virus, and the presence of specific blocking factors in the serum.

Significance to Biomedical Research and the Program of the Institute: The expression of MMTV in non-mammary gland tissue and the effect of cocarcinogens on the expression of MMTV is important to understanding the pathogenesis of virally-induced breast cancer and to prospects for the prevention or control of this disease.

Proposed Course: To continue the activities described at the current level.

Date Contract Initiated: April 26, 1974

Current Annual Level: $162,562

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP6-1053)

Title: Studies to Determine a Viral Involvement in Feline Mammary Carcinoma

Contractor's Project Director: Dr. Nurul H. Sarkar

Project Officer (NCI): Dr. John Dahlberg

Objective: To employ techniques of virus isolation, cell culture, immunology and biochemistry to elucidate a possible viral etiology in feline mammary carconoma.

Major Findings: During this first contract period, 43 feline mammary tumors were obtained. Electron microscopic, immunologic, biochemical and tissue

culture techniques were used to search for a putative feline mammary tumor virus in these tumors. Intracisternal type A particles were observed in 5 of the 29 tumors examined to date. Immunological studies using polyvalent anti-mouse mammary tumor virus (MMTV) serum detected reactive antigens in 5 of the feline tumors. A monospecific serum to MMTV gp34 reacted with a feline mammary tumor containing a large number of intracisternal type A particles, but the specificity of this reaction remains to be evaluated. Two feline mammary tumor cell cultures were established. One with epithelial morphology was characterized. Cells from this culture produced a tumor when injected into nude mice. The culture fluids had a low level of RNA-directed DNA polymerase (RDDP) activity. Biochemical studies showed that 3 feline mammary tumors contained RDDP.

Significance to Biomedical Research and the Program of the Institute: Currently, study of virus-induction of mammary carcinoma has been confined to mouse systems. Evidence suggests a virus may be associated with human breast cancer. Studies to extend knowledge of viruses in relation to this disease to other animal species than the mouse will yield greater insight into factors which bear upon the human disease.

Proposed Course: Cocultivation studies will be initiated to attempt to isolate and characterize virus from feline mammary tumor cells.

Date Contract Initiated: June 27, 1976

Current Annual Level: $150,000

TEL-AVIV UNIVERSITY (NO1-CP5-3517)

Title: Vaccination of Mice with Cell-Free Antigens Derived from MMTV and A Particles

Contractor's Project Director: Dr. Asher Frensdorff

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To test whether it is possible to immunize mice against mouse mammary tumor virus (MMTV) without exposing them, in the process, to structurally intact virus. To investigate whether immunization of mice with individual, purified structural proteins from MMTV can decrease or abolish their susceptibility to spontaneous as well as virus-induced mammary tumors. To isolate and purify the structural proteins under the mildest possible conditions to ensure conservation of the native conformation of the proteins. To immunize in such a way as to (a) increase as much as possible the immunogenicity of the proteins of which only very small doses are to be infected and (b) stimulate a cell-mediated, rather than humoral, immune response.

Major Findings: A goat antiserum was produced which is monospecific to gp52 by immunodiffusion and by radioimmunoprecipitation analysis and which does not react with antigens of murine leukemia virus (MuLV). A procedure was developed to isolate gp52 from disrupted MMTV at close to maximal yields; by affinity

chromatography, using a sugar-specific lectin purified from lentils, 16% of the protein can be recovered as gp52 from disrupted MMTV. PAGE-SDS gels of such preparations were virtually free from other proteins. Immune precipitation of the isolated protein with monospecific anti-gp52 provides an immunogenic vaccine. Since the antiserum used did not cross react with antigens of MuLV, it was probably not essential to use very pure MMTV as the initial source.

Each antiserum was characterized as to its specificity by double diffusion in agar gels at large antibody excess, against a battery of test antigens.

Significance to Biomedical Research and the Program of the Institute: These studies identify those virus fractions which have protective capability. Work with this virus is especially important since it provides a model system for solid tumor immunity which may be somewhat different from the existing leukemia models. Because of the demonstration of infectivity by viral nucleic acids, an immunizing fraction free of nucleic acid appears to be greatly desirable.

Proposed Course: This contract erminated on June 23, 1976, and the effort has been readvertised.

Date Contract Initiated: October 24, 1974

Current Annual Level: $31,124

TEXAS, UNIVERSITY OF (NO1-CP4-3370)

Title: Immunological Studies on Animal and Human Breast Cancer

Contractor's Project Director: Dr. James M. Bowen

Project Officer (NCI): Dr. Meera Paranjpe

Objectives: (1) To apply a variety of immunological methods for detection and characterization of virus and tumor-associated antigens and their antibodies in mice with mammary cancer; (2) to apply similar methods to a study of antigens in cells derived from human breast cancer patients; and (3) to determine by these methods whether viruses similar or related to mouse mammary tumor virus may be associated with the human neoplasm.

Major Findings: Immunofluorescence, complement fixation, and humoral cytotoxicity assays were applied to detect antibodies in mouse and human sera to establish patterns of reactivity and determine nature, distribution, and specificity of antibodies to both homologous and heterologous breast tumor-associated antigens. Observations made by application of these assays were subsequently extended, confirmed, and further analyzed by other techniques, primarily immunoelectron microscopy and radioimmunoassays.

Studies showed that mice of all strains studied are immunocompetent against mouse mammary tumor viruses (MMTV) antigens. Mice bearing MMTV-producing mammary tumors, whether spontaneous or transplanted, produce antibody to MMTV,

predominantly anti-virion envelope antibodies. Adult high-mammary tumor strain mice without tumors also have MMTV-specific serum antibodies, but most animals produce antibody to internal virion components rather than to virion envelope antigens. Young mice of all strains tested were free of detectable anti-MMTV antibodies and developed an immune response when injected with virus or isologous tumor cells.

Patients with breast cancer have serum antibodies to a variety of breast tumor-associated antigens. Sera of many patients reacted with MMTV-producing mouse mammary tumor cells. Reactivity could only be removed by absorption with MMTV preparations. Cells of some human breast tumor cultures produced antigens which reacted with specific anti-MMTV sera.

A number of cell lines of mouse or human breast tumor origin were studied using heterologous antisera against oncornaviruses or their components. An antiserum prepared against MMTV gp52 of C3H mice gave positive reactions of low titer against the MCF-7 human breast cancer cell line. An antiserum against whole disrupted MMTV from A/Dm strain mice reacted with MCF-7 cells and cells of one human breast cell line. MCF-7 was also positive in some passages. A high titer antiserum against whole disrupted Rauscher leukemia virus particles also reacted with MCF-7 cells. The significance of any of these reactions with heterologous antisera remains to be determine by further work with more sensitive and specific methods.

Significance to Biomedical Research and the Program of the Institute: Mammary tumor viruses have been shown to be etiologically related to breast cancer in mice, and there is some evidence suggesting the presence of virus in human mammary cancer. This study was initiated to clarify the nature of the immunological cross-reactivity between mammary cancer in humans and virus-induced cancer in mice.

Proposed Course: This contract erminated on October 31, 1976.

Date Contract Initiated: June 28, 1974

Current Annual Level: $84,783

SUMMARY REPORT

(2) CLINICAL STUDIES SECTION

July 1, 1976 through September 30, 1977

The Clinical Studies Section identifies, develops and coordinates those contract research efforts within the Virus Cancer Program most directly applicable to human disease. Although the specific mission involves implementation of studies which are directly related to the etiology, diagnosis, prognosis and control of human malignancies, it also includes animal models designed to investigate diagnostic, preventive and/or therapeutic procedures which have potential for human application. In a similar manner, clinical studies using epidemiologic probes to determine the etiology of human malignancies include those which are fundamentally human in scope as well as natural history studies of disease patterns in animals, such as Marek's disease, feline leukemia, and murine leukemia.

Among the animal models studied, considered relevant to the human situation, the existence of vertically-transmitted leukemia viruses in outbred mouse populations continues to be investigated in wild mice at the University of Southern California. In addition to a neurotropic, leukemogenic ecotropic type C virus reported previously, an amphotropic, leukemogenic type C virus has been identified, which, it is suggested, may be the parental virus for the laboratory strains of ecotropic and xenotropic viruses currently investigated by many groups. At USC, investigators demonstrated the presence of a type C virus in rat embryo cells, which accelerates cell transformation by chemical carcinogens, though by itself it does not seem to be leukemogenic or sarcomagenic under experimental conditions.

The feline leukemia system is another naturally occurring model of considerable relevance. Conditions for the infection of kittens have been investigated at Ohio State University (OSU). A strong age-related susceptibility was demonstrated in cats to both feline leukemia virus (FeLV) and feline sarcoma virus (FeSV), and a correlation was demonstrated between the presence of FeLV group-specific antigen in blood cells and infectious virus in the plasma. At USC, studies on feline leukemia have been carried out under field conditions, and horizontal spread of FeLV was confirmed. The presnce of FeLV RNA, and the p30 and gp70 virus antigens was shown to be predictive for lymphoma development in young but not old cats.

At Rush-Presbyterian-St. Luke's Medical Center (RPSL) and at USC, studies were made on the presence and pathogenicity of Epstein-Barr (EBV)-like viruses in marmosets and other primates. At USC, an EBV-like herpes virus was isolated from a cell line originated from an orangutan with spontaneous myelomonocytic leukemia.

The natural presence of such viruses in susceptible animals not only has importance in tumor prediction and epidemiology, but also may provide an important marker in prophylaxis, diagnosis and therapy. At Microbiological

Associates (MAI), much effort has been focused on defining the ecotropic type C viruses in mice of various strains and the relationship of such viruses to spontaneously-arising and chemical carcinogen- and X-ray-induced tumors, with an aim to development of prophylactic immunization techniques. At the Fred Hutchinson Cancer Research Center, murine leukemia virus (MuLV)-related cell surface antigens have been demonstrated on both embryonic and methylcholanthrene-induced tumor cells derived from BALB/c mice. Such virus gene expression can occur in the absence of whole virus production.

An intriguing and very puzzling successive series of epidemics of lymphomas occurred in a hamster colony, housed at the University of Tennessee. The lymphomas were obviously due to horizontal transfer of an infectious agent, the nature of which has not yet been elucidated. Previously, a rhesus monkey colony held at the University of California at Davis exhibited a high lymphoma incidence, which was confined to four rooms. The etiologic agent was not identified.

In humans, no clear viral etiology for any tumor has been demonstrated as yet. Strong evidence suggests that EBV is closely associated with Burkitt's lymphoma and with nasopharyngeal carcinoma (NPC). Prospective and retrospective epidemiological and immunological studies through the International Agency for Research on Cancer (IARC) are continuing on the role of EBV and other factors in these diseases. Other studies on EBV are being carried out by Children's Hospital of Philadelphia and Litton Bionetics Inc., using both seroepidemiological and cell-mediated immunological techniques.

A number of epidemiologic studies by the Center for Disease Control (CDC), the Fundacion para la Educacion Superior (FES), Hebrew University, University of California at Los Angeles and USC, have attempted to correlate such factors as interpersonal and animal contacts, race, genetic traits, previous virus infections and immunological status with the development of leukemia, lymphomas, and other cancers in humans. No clear associations were observed between cancer and/or animal contacts. Certain clear associations were obtained at UCLA between HLA type and susceptibility to such tumors as lymphocytic leukemia and prostate cancer. At USC some evidence was obtained for associations between certain endocrine dyscrasias and breast cancer, and environmental carcinogens and NPC. Biotech Research Laboratories investigated possible correlations between susceptibility of cells from normal and high cancer risk individuals to transformation by simian sarcoma virus or SV40; technical difficulties precluded any definite conclusions.

Circumstantial evidence for a transmissible factor in the etiology of some tumors is found in the increased specific immune reactivity of close associates and relatives of cancer patients, as observed at the Fred Hutchinson Cancer Research Center. The Boston Hospital for Women provided serial serum specimens and pathological data from women with genital herpesvirus type 2 infections, cervical dysplasia and cervical carcinoma. These should permit retrospective cohort studies on the possible role of herpesvirus type 2 in cervical neoplasia.

Investigators at the Massachusetts General Hospital, Stanford University, and at other institutions are examining malignant cells from patients with lymphomatous diseases for the presence of virus related to simian sarcoma virus. A report from Stanford University described the isolation of a type C virus from a human histiocytic lymphoma cell culture. Immunologically and biochemically this agent is related but not identical to the simian sarcoma virus. If the disease relationship of this virus is established, immunological probes against it could be of great use in the diagnosis of human lymphomas and could possibly be of value in therapy.

A number of studies on methods of prophylaxis against the development of virus-induced neoplasia have been carried out. The Fred Hutchinson Cancer Research Center reported some evidence obtained in mice for inhibition of the development of chemical carcinogen-induced tumors by active and passive immunization against embryonic and murine leukemia virus antigens. Similar immunoprophylactic experiments have been carried out in mice by MAI, using active or passive immunization against Rauscher or Gross viruses prior to induction of leukemias or other tumors by x-irradiation or chemical carcinogens. Investigators at USC demonstrated the efficacy of passive immunity against congenitally-acquired lymphoma in susceptible mice.

The presence of natural immunity against a number of oncogenic viruses was observed in mice. The Fred Hutchinson Cancer Research Center has investigated natural immunity of I-strain mice, which are resistant to oncogenic virus infection. Antibodies against MuLV in multiparous female mice, presumbably as a result of sensitization to MuLV, were expressed in the fetus. At the University of Tennessee, the presence of antibody to fetal antigens has been demonstrated in multiparous hamsters which renders them resistant to SV40-induced oncogenesis. Anti-MuLV antibodies have been shown in non-leukemic AKR mice at Mount Sinai School of Medicine, indicating that these animals can respond immunologically to the antigens of their endogenous virus.

A number of studies have been carried out in animal models for the development of viral vaccines against tumor development. Merck and Co., Inc. has been evaluating purified viral protein vaccines as practical immunogens against herpesvirus type 2 infection in guinea pigs, since only a non-nucleic acid-containing virus vaccine would be acceptable for eventual human use against genital herpes infections, and for possible prophylaxis against cervical and other cancers thought to be associated with prior herpes infection. Merck also is testing virus protein vaccines against FeLV infection in cats. In the course of their studies on feline leukemia, investigators at Ohio State University are evaluating virus-specified cell membrane and soluble virus protein vaccines for effectiveness in prevention of virus infection or tumor development.

In immunosuppressed individuals, following kidney transplantation, the risk of development of reticulum cell sarcomas and other tumors, as well as generalized virus infections, is far greater than normal. Based upon prior studies at Massachusetts General Hospital which showed interferon to prevent tumor virus activation in mice receiving grafts, prophylactic therapy

experiments using human interferon have been initiated in renal transplant patients at that institution. Results are not yet available, but this may serve to demonstrate the potential of interferon in controlling activation of covert virus infections in such individuals.

At Mount Sinai School of Medicine, the concept that cytoreductive therapy, without virus destruction, may leave the host susceptible to reinduction of tumor has led to a number of experimental and clinical models to test antiviral therapy concomitant with antitumor therapy. Using neuraminidase-treated syngeneic and allogeneic tumor cells following cytoreductive chemotherapy or tumor ablation, evidence for permanent cure, as well as prophylaxis against primary tumor development, was obtained in both the murine AKR lymphoma and C3H mammary tumor systems. Antiviral agents, such as interferon, virazole and possible phosphonoacetic acid, can substitute for the treated cells. Of particular importance is that in human acute monocytic leukemia, chemo-immunotherapy, using neuraminidase-treated allogenic myeloblasts has produced a remarkable increase in the observed duration of remission.

The investigation of virus-associated or other tumor antigens, or the presence of virus-assoicated antibodies or cellular reactivity, as diagnostic and prognostic tools for the presence of cancer is becoming increasingly important. At Children's Hospital of Philadelphia and IARC, serological studies have demonstrated correlation of immune status to EBV-related antigens with risk of primary Burkitt's lymphoma and/or relapse and with the clinical status of nasopharyngeal carcinoma. Studies have also been carried out at Litton Bionetics, Inc. and George Washington University on cellular reactivity to EBV-related antigens in infectious mononucleosis, lymphomas and nasopharyngeal carcinoma, with clear correlation with disease status in some cases. At UCLA, studies of lymphocyte cell surface markers are promising as a method of characterizing certain childhood leukemias for prognosis and therapy. In Hebrew University, the use of the fluorescent con-A probe as a diagnostic tool for Hodgkin's disease is proving of interest. Studies are in progress to determine whether the type C virus identified in cells from a case of Hodgkin's disease at Stanford University may be of use as a diagnostic probe.

Apart from the work at Mount Sinai School of Medicine, mentioned previously, a number of contractors have carried out experiments in possible therapy techniques, both experimental and clinical. At University of Alabama, experiments were conducted to define the conditions of both direct cell-mediated immunity agaomst virus-induced tumors and antibody-dependent cellular cytotoxicity. At MAI, experiments are in progress investigating the conditions necessary to induce immunity to "virus-free" syngeneic tumor challenge by means of viral antigens and virus-infected tumors. At the University of Tennessee studies are being undertaken on the relationship of virus-specified and fetal antigens in the induction of tumor transplant immunity in hamsters and the immunizing conditions for the development of optimum tumor resistance. Using antigenic deletion techniques, two SV40-induced tumor cell lines have been produced in which fetal antigens are no longer expressed. Attempts are being made to purify the SV40-specific surface antigens. In the Fred Hutchinson Cancer Research Center investigations were made on the role of virus-associated antigens in tumor transplant immunity against both chemical carcinogen- and virus-induced tumors,

and on the role of natural immunity in protection against tumor challenge. Of particular interest is a new adoptive immunotherapy model in which syngeneic or allogeneic macrophages, which have previously been allowed to react with tumor antigen, are used to sensitize syngeneic lymphocytes *in vitro* such that they are able to react *in vivo* in a specific Winn Test.

CLINICAL STUDIES SECTION

Dr. David H. Lavrin, Acting Head

ALABAMA, UNIVERSITY OF (NO1-CP4-3394)

Title: Immunologic Studies on the Relationship of Embryonic Antigen to Virus-Induced Tumor Antigens

Contractor's Project Director: Dr. Eddie W. Lamon

Project Officer (NCI): Dr. Paul Peebles

Objectives: Investigation of the cellular immune responses of mice to embryonic and virally-induced antigens of tumor cells by analyses in vitro to determine the magnitude of the response to each class of antigen, the nature of the effector cells, and the mechanisms involved in specific cytolytic responses.

Major Findings: The function and significance of embryonic and virally-induced tumor antigens was studied in terms of the cellular immune reponse to such antigens.

In the microcytotoxicity test, it was found that hyperimmunization of C3H mice with mitomycin-killed syngeneic polyoma virus-transformed cells results in a delicate balance between specific and non-specific immunogenicity and tolerogenicity. At 10^6 cells for the final immunizing dose, maximal specific anti-polyoma immunity was observed, with no H-2 restriction of specificity. At 10^7 cells, specific anti-polyoma cytotoxicity was lost, and strong cytotoxicity for SV40-transformed cells emerged. At 10^5 cells final immunizing dose, no cellular cytotoxicity was detected. In fact, lymphocytes from some animals stimulated growth, not only of the polyoma-transformed target cells but also those transformed by SV40 and MSV as well as untransformed 3T3 cells and primary embryo fibroblasts. Immunization against embryo fibroblasts in some cases also produced a similar non-specific stimulation of target cell growth. The possibility that this is a specific immunostimulation by common embryonic antigen(s) is being investigated, as also is the possibility that the non-specific immunity detected following the 10^7 immunizing dose is due to sensitization against common embryonic antigens.

Studies were made on the nature of the effector cells involved in microcytotoxicity. They are not macrophages since iron powder-magnet treatment does not decrease their activity. The possibility that specific cytotoxicity is restricted to T-cells, whereas cross-reacting cytotoxicity is due to B-cells, is being investigated.

Since it is probable that this is a major factor in tumor immunity, antibody-dependent cellular cytotoxicity (ADCC) in polyoma tumor immunized

mice was investigated. It was demonstrated that this is a specific phenomenon, with no reaction against SV40 or MSV-transformed cells. Splenocytes, thymocytes and lymph node cells were all activated by serum from polyoma tumor-immune mice. Sera from mice immunized with embryo fibroblasts were broadly cross-reactive--least against the polyoma tumor cells used in these studies.

Sera from MSV tumor-regressor mice, both IgM and IgG, were active in specific ADCC. However, whereas IgM was active with splenocytes, lymph node cells and thymocytes, IgG was effective only against the first two. Thymus cells, active with IgM, were found to be cortisol-resistant. In addition, cortisol-resistant thymocytes entered into significant cytotoxicity with IgG-coated tumor cells. The existence of IgM Fc receptors, distinct from IgG and C3 receptors, on lymphoid cell populations active in ADCC was confirmed.

Significance to Biomedical Research and the Program of the Institute: The determination of the association between virus transformation and the expression of fetal antigens was undertaken to obtain better understanding of the cross-reactivity between viral antigens and tumor-associated antigens which might occur in humans. Characterization of these antigens may lead to more specific assays relevant to tumor etiology and diagnosis. In addition, an understanding of the cellular and humoral responses of the host to these antigens will contribute information which may be important to understanding the mechanisms of viral oncogenesis and to the feasibility of using tumor antigens in immunological control experiments against virus-induced tumors.

Proposed Course: This contract terminated July 14, 1977.

Date Contract Initiated: June 28, 1974

Current Annual Level: $56,500

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP4-3365)

Title: Transformation of Fibroblastic Cells Derived from Patients Afflicted with Genetic Diseases, Hodgkin's Disease (HD), Nasopharyngeal Carcinoma (NPC), and from Donors of Multiple Case Cancer Families

Contractor's Project Director: Dr. Robert Ting

Project Officer (NCI): Dr. Steven Tronick

Objective: To explore human genetic variation in susceptibility to cell transformation by oncogenic RNA and DNA viruses and help identify high cancer risk groups.

Major Findings: A total of 193 cell cultures were tested for their susceptibility to simian sarcoma virus (SSV) transformation and SV40 T-antigen induction. In each experiment, whole human embryo (WHE) cells were used as standard control cells. The results obtained with the first group of cells,

which were from various laboratories, indicated that cells derived from normal persons were 4 to 8 times more resistant to SSV transformation than cells derived from leukemia patients and patients with other cancer or genetic diseases. However, the results obtained with the second group of cells supplied by the Jewish Hospital in Brooklyn did not follow the same pattern. Since most of these two groups of cell cultures were supplied by other laboratories, the method of establishing and maintaining these cultures were not known an effort was then undertaken to establish cell culture from tissue specimen at Biotech. The results with cell cultures (91) established by the contractor showed that there was no significant difference among the normals, leukemia and lymphoma patients, and the other cancer patients. The rate of cell growth was observed to greatly influence the frequency of transformation by SSV and to a certain extent SV40 T-antigen induction. Therefore, one could not readily assess whether the degree of susceptibility to SSV transformation can be used to identify groups of individuals that are more susceptible to cancer development that individuals of the general population, unless the growth rate of each individual cell culture is carefully controlled.

Significance to Biomedical Research and the Program of the Institute: Genetic factors are known to affect susceptibility to cancer development in laboratory animals. This project was initiated to investigate one approach to identify such groups in the human population and develop methods to measure the degree of susceptibility.

Proposed Course: This project terminated on December 31, 1976.

Date Contract Initiated: June 28, 1974

Current Annual Level: $54,000

BOSTON HOSPITAL FOR WOMEN (NO1-CP4-3379)

Title: Retrospective Cohort Study for Cervical Neoplasia and Herpes Simplex Virus Type 2

Contractor's Project Director: Dr. Lorna D. Johnson

Project Officer (NCI): Dr. John Pearson

Objectives: To identify patients who had or developed cervical neoplasia (atypia, dysplasia, carcinoma in situ or invasive cancer) and other cancers together with matched controls among a population of 12,708 women in the Collaborative Study for Cerebral Palsy (CSCP) who were screened for cervical neoplasia by Papanicalaou smears and who had serum samples taken during the period from 1959 through 1965 at the Boston Hospital for Women. To provide current historical, cytologic and histopathologic data and serum samples from these women for comparison with previous samples on file at the National

Institute for Neurological and Communicative Disorders and Stroke (NINCDS). To provide similar data and serum samples from patients with cervical neoplasia and genital herpes who are currently attending the Special Cervical Clinic at the Boston Hospital for Women.

Major Findings: A total of 3,067 serum samples together with detailed histories, Papanicalaou smears and colposcopic examinations have been obtained from patients with cervical neoplasia, other cancers, genital herpes infection and vaginal adenosis and from control women. Serum samples have been aliquoted and sent under code to the NIH. One thousand nine of these patients had one or more serum samples taken during the years 1959 through 1965. These sera were banked at the NIH and are available for comparison with current samples.

Pertinent data are computerized.

Serum samples from 40 patients, from whom herpesvirus hominus (HVH) types 1 and 2 were isolated, were assayed for HVH antibodies by microneutralization, indirect hemagglutination and indirect immunofluorescence. Data are to be submitted for publication.

Approximately 2,800 sera have been tested for antibodies to HVH_1 and HVH_2 by the indirect hemagglutination assay. Data are being processed for correlation between the stages of cervical neoplasia and herpesvirus hominis type 2.

Significance to Biomedical Research and the Program of the Institute: The unique population from which the specimens are being drawn permits a "prospective"study to achieve more information concerning the relationship between HSV-2 and cervical cancer.

Proposed Course: This contract terminated February 28, 1977.

Date Contract Initiated: June 4, 1974

Current Annual Level: $65,000

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP5-3504)

Title: Studies on the Viral Etiology of a Malignant Lymphoma Outbreak in Rhesus Monkeys

Contractor's Project Director: Dr. JaRue Manning

Project Officers (NCI): Dr. Paul Peebles
Dr. Kenneth Takemoto (Assistant)

Objectives:

To study the epidemiology and suspected viral etiology of an outbreak of spontaneous malignant lymphoma in rhesus monkeys at the California Primate Research Center (CPRC) and to compare the findings of these studies with those of others on viral-induced lymphoma of nonhuman primated and spontaneous lymphoma of man. The specific aims of this research proposal are: (1) to conduct a detailed epidemiological analysis of the lymphoma outbreak; (2) to examine serum samples from lymphoma-bearing and high risk monkeys for antibodies to simian oncornavirus and Epstein-Barr virus (EBV) and virus-associated antigens; (3) to detect and characterize oncornavirus and herpesvirus-like particles and their associated antigens in spontaneous lymphoma tissues; (4) to propagate lymphoma-derived cells in tissue culture and screen for the presence of herpesvirus-like and oncornavirus-like particles by immunological and biochemical methods.

Major Findings:

Epidemiologic studies have shown that rhesus monkeys developing lymphoma were resident in one or more of four animal rooms during a period between

September 1968 and April 1969. There was no significant association between duration of stay and development of disease. Although the incubation of the disease appeared to be normally distributed suggesting a single event, no event which may have precipitated the disease has yet been discovered. Immunologic studies have confirmed earlier findings of elevated serum protein levels in lymphoma-bearing rhesus monkeys. The precise relationship between the increase in serum proteins and the occurrence of lymphoma is unclear at this time. All rhesus houses at the facility were being given Isoniazid (INH) prior to and during the outbreak. Continuing studies have now shown that INH administered to healthy adult rhesus monkeys depresses their immunologic capacity as measured by a reduction in the T-cell mitogen stimulation index and delayed type hypersensitivity. INH treatment therefore may have placed all rhesus monkeys at a high risk and only those monkeys exposed to or infected with the inducing agent(s) subsequently developed lymphoma. Exhaustive virologic studies of lymphoma tissues and sera from tumor-bearing monkeys has demonstrated the presence of several different viruses, however, none of the findings thus far show a consistent correlation between the detection of a virus or antibodies to viral antigens and the presence of disease.

Significance to Biomedical Research and the Program of the Institute:

The spontaneous outbreak of malignant lymphoma in macaques under study is the first such outbreak in nonhuman primates recognized in this country. A number of the observations made on the disease in the rhesus monkey showed a parallel with lymphoma in man, particularly Burkitt's lymphoma. As in humans, the etiology of this neoplasm in the rhesus monkey is unknown. Studies on Burkitt's lymphoma indicated that both a herpesvirus and an oncornavirus mignt be involved. Both may be involved in the outbreak of lymphoma in non human primates being studied. Obtaining critical information on the etiology and immunobiology of this outbreak of malignant lymphoma in rhesus monkeys may appreciably enhance our understanding of lymphoma in man.

Proposed Course: This contract terminated March 22, 1977.

Date Contract Initiated: September 23, 1974

Current Annual Level: $66,000

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP4-3211)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer

Contractor's Project Director: Dr. Paul I. Terasaki

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the cellular components of the immune response to cancer and its interaction with antibodies in terms of resistance or susceptibility to cancer; (3) to study the immune response of cancer patients and normals to different viruses and virus-induced antigens; (4) to examine the relationship of genetic markers (HL-A and others) for linkage to the incidence of cancer in families; and (5) to characterize B-lymphocyte alloantigens and to determine their expression in cell hybrids and changes in cells following virus infection or transformation.

Major Findings: HL-A typing 4014 cancer patients and 3,896 controls between 1970-1975 noted a weak association of different HL-A types with certain cancers. An independent series of 2,005 new patients has confirmed variances in the incidence of specific HL-A types in lymphocytic leukemia and prostatic cancer. Indications have also been obtained for minor variances in breast, cervical, esophageal, laryngeal, liver, lung, ovarian and gastric cancer; also for lymphosarcoma, lymphoma, myelocytic leukemia and Hodgkin's disease. Family studies are in progress. If a susceptibility gene exists, it is probably located at some distance from the HL-A locus. It is also felt that if HL-A type affects cancer risk, it is more likely to affect cancer early in life.

Studies have been initiated on a possible association between B-lymphocyte type and cancer. A major effort is being made to identify and characterize B-lymphocyte alloantigens in man.

A study between HL-A type and immune response to influenza virus has indicated a decreased response in HLA-BW16 individuals. In response to rubella vaccine in children, clear differences were detected between high and low responders with respect to certain HL-A types. In Hodgkin's patients, a significantly lower titer to VCA and EA, not detected in non-cancer controls, was found in those with HLA-B27 antigen.

In transplant patients, those receiving kidneys from well-matched HL-A-identical donors develop skin cancer at a higher rate than those receiving HL-A incompatible grafts. The possibility is being considered that with compatible siblings oncogenic virus transfer from graft to host may be more effective. In contrast, in well-matched sibling transplants reticulum cell sarcoma is not found.

Sera from leukemia/lymphoma patients were found to possess a depressed lymphocyte-dependent antibody activity. Similarly, sera from the majority

of cancer patients were found to possess inhibitory activity against antibody-dependent cellular cytotoxicity. This may prove a useful test for immunological function in cancer patients.

During the past year a considerable effort has been made to correctly type cultured human lymphoblastoid cell lines. Until recently these have been likely to yield false positives, mainly due to impurities in the typing reagents. These are now being purified. Already, the ability to characterize cell lines has led to identification of contamination and errors in the designation of a number of cell lines.

A study is being carried out on HL-A and B-cell antigen expression in cell hybrids and EBV-infected cell lines. These are being used to determine successful hybridization and to indicate possible phenotypic changes following viral infection or transformation. Some new B-antigens have been detected; in other cases total loss of certain B-antigens has been demonstrated.

Lymphocyte cell surface markers for different reagents (e.g. T-lymphocyte markers, receptors for sheep erythrocytes, Fc and complement; new receptors, or lack of receptors) have been used to characterize a number of childhood leukemias. This has been useful for determining prognosis, and for indicating appropriate therapy. In addition, B-antigen, group 2, found in 22% of the general population, has not been detected in over 50 leukemic patients.

Specific granulocyte antigens, characteristic of myeloid cells, have been observed on a chronic myelogenous leukemia cell line. This may serve as a diagnostic test for such leukemias.

Studies have continued on the specificity of natural cell-mediated immunity in normal individuals and cancer patients. Both specific and non-specific reactivity were observed. The effector cells in natural cell-mediated cytotoxicity were 'N' cells, the specificities of which were directed by many specific antibodies, attached to their Fc receptors. The aggregate effect was for wider (non-specific) or narrower (specific) reactivity of a given cell population.

Both qualitative and quantitative differences were detected between target cells before and after treatment with viruses and chemicals.

Significance to Biomedical Research and the Program of the Institute: The identification of disease susceptibility genes is of great importance to studies on the etiology of cancer. In family studies, linking genetic type and immunologic response to tumor- and virus-associated antigens may help to determine which humans are more likely to develop specific tumors as well as which abnormal immune responses to certain viral antigens are closely linked to the tumors. The investigators have been involved in studies indicating that individuals with certain HL-A types have a diminished response to certain viral antigens, particularly of influenza and rubella viruses. Studies in progress may help to determine whether certain groups of people have an appropriate response to EBV or other suspected tumor viruses. B-cell typing studies are likely to provide useful techniques for characterizing lymphoid cell lines, studying antigenic expression and modulation following virus infection or

transformation, and in the diagnosis, prognosis and treatment of various forms of leukemia.

Proposed Course: Studies on familial cancer will continue and, in addition to HL-A typing of family members, lymphocyte defined antigens will be measured. An attempt will be made to obtain immunologic profiles on normal individuals in cancer families. The cytotoxicity assay will be applied to animal systems attempting to relate the cellular and humoral immune response to oncogenic viruses.

Date Contract Initiated: July 12, 1971

Current Annual Level: $285,000

CENTER FOR DISEASE CONTROL (NO1-CP4-0202)

Title: Epidemiologic Studies of Leukemia and Related Diseases

Contractor's Project Director: Dr. Clark Wright Heath, Jr.

Project Officers (NCI): Dr. Paul Peebles
Mr. Roger Connelly (Assistant)

Objectives: To relate epidemiologic studies of leukemia and related diseases to laboratory assays pertinent to the viral etiology of cancer by: (1) ad hoc field investigations of community clusters of cancer cases, unusual viral illnesses, and/or animal tumor-associated cases; (2) multiple-case family studies; (3) cohort study of heterophile-positive infectious mononucleosis (IM) cases with respect to subsequent cancer risk; and (4) epidemiologic study of interpersonal contact in relationship to leukemia and lymphoma incidence.

Major Findings: Work under this contract and its predecessor, VCL-42 covers a 9-year period and has involved the following activities (in rough chronologic order): (1) field investigations of community clusters of cancer cases, (2) investigations of human malignancies in association with animal contacts, (3) studies of multiple case cancer families, (4) surveillance of leukemia, lymphoma, and childhood cancers in selected populations, (5) collection of serum specimens from patients and family members for laboratory research purposes at NCI and elsewhere, (6) collection of tissue specimens for cell culture at CDC and provision of such cultures to NCI-collaborating investigators, (7) statistical studies of time-space clustering of leukemia cases, (8) epidemiologic study of the effect of prenatal influenza on childhood leukemia-lymphoma risk, (9) epidemiologic study of cancer risk in heterophile-positive infectious mononeucleosis (IM) cases at selected U.S. colleges, (10) epidemiologic study of high school interpersonal contact as a possible risk factor among young adult cases of leukemia and lymphoma in Connecticut, and (11) seroepidemiologic study of bovine leukemia virus in dairy herds and herdsmen and their families.

Those portions of work concerned with the epidemiologic question of time-space clustering have produced largely negative data, both from systematic statistical analyses of case distributions and from ad hoc studies of apparent clusters. Certain studies, however, did produce suggestive evidence of clustering among childhood leukemia cases. Efforts to develop this evidence further, through virologic laboratory studies of selected clustered cases, have as yet been nonproductive.

Multiple-case cancer family studies produced evidence in two instances of possible immune deficiency related to lymphoreticular tumor risk. Serologic studies involving several families suggested a possible relationship between antibody against EBV and risk of leukemia/lymphoma.

Other studies directed at hypotheses of human oncogenic infection have produced a variety of results. The study of maternal influenza in relation to childhood cancer risk was essentially negative. The study of cancer following IM, while too small to be conclusive, suggested a possible relationship of IM with subsequent HD. The recent study of high school contacts in Connecticut showed a 2-fold increase in risk of HD but not other forms of hematologic malignancy. The bovine leukemia seroepidemiologic studies showed no evidence of human infection.

Significance to Biomedical Research and the Program of the Institute: Epidemiologic studies such as these are important in order to resolve the question of whether infectious agents are involved in the etiology of human cancer, and, if so, whether animal contacts, age-related susceptibility and other virus infections play any role.

Proposed Course: This contract terminated September 30, 1976

Current Annual Level: $110,000

CHILDREN"S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Seroepidemiology of Epstein-Barr Virus (EBV)

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Berge Hampar

Objectives: To investigate the relationship between EBV and human cancer. Studies on this contract include: (1) improvement of existing, and development of new techniques for the detection of EBV-related antigens and titration of corresponding antibodies; (2) search for methods to measure cell-mediated immune reactions in EBV-associated diseases, and if successful, their application to detection of blocking (tumor enhancing) factors; (3) determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases providing prognostic information and support for a causal relation of EBV to given human malignancies; and (4) attempts to

improve yields of infectious virus from present sources and search for new sources in efforts to obtain potent EBV preparations and to compare biologic properties of different isolates.

Major Findings: Burkitt's lymphoma (BL): The prognostic implications of antibodies to the R component of the early antigen (EA) complex were confirmed in 141 patients from Ghana. The anti-R titers reflected to some extent the stage of the disease. After a year's sustained remission, patients with anti-R titers of >1:80 showed a 6-fold greater probability of relapse than those with lower titers. Anti-EBNA titers of Ghana patients showed an inverse relation to dermal reactivity with Raji antigen, whereas in Uganda a direct relation to skin reactions with recall antigens became apparent.

Detection of EBV, DNA and/or EBNA+ cells in biopsies of BL (not found in other lymphomas) correlated well with the EBV-related serology; DNA tests were more dependable than EBNA tests, presumably due to escape of antigen from nuclei under processing. A focus of 3 cases of BL was discovered in rural Pennsylvania; 2 were close friends. All were serologically EBV-associated; EBV DNA and/or EBNA+ cells were found in tumor specimens available from 2. Based on genome and serologic studies between 15-25% of American cases appear to be EBV-associated.

Nasopharyngeal carcinoma (NPC): The long-term follow-up study of Hong Kong patients is near completion. Patients responding well to therapy show gradual declines and ultimate loss of both IgG and IgA antibodies to the de-component of the EA complex and of virus capsid antigen (VCA)-specific IgA. In patients with residual or recurrent tumors antibodies remain elevated or increase, and a few may show VCA-specific IgM antibodies. Several patients presently well with intermediate patterns require further observation. Two separate studies are in progress correlating EBV-related serology with evidence of EBV DNA and/or EBNA+ cells in biopsies of NPC or other carcinomas. Only anaplastic or poorly differentiated NPC revealed EBV genomes, whereas well differentiated NPC and other carcinomas thus far were negative. Studies on American NPC patients turned problematic since on assortment, according to various criteria, some groups became too small for valid comparisons.

Hodgkin's and other lymphomas: In support of the notion that immunosuppressive diseases may activate persistent EBV infections, some patients with these diagnoses revealed VCA-specific IgM antibodies at titers up to 1:320. It is not yet known whether the IgM antibodies are preceded by declines in T cell functions and followed by increase in IgG antibodies.

Infectious Mononucleosis: Efforts are made to correlate EBV-specific cell-mediated immune reactions, observed early in IM, with the time of emergence of anti-EBNA. Colony formation by PHA-stimulated lymphocytes of IM patients (but not of healthy donors) is inhibited by incorporation of autologous plasma in the agar. The nature of this reaction is under study.

Significance to Biomedical Research and the Program of the Institute: The primary purpose of these studies is to aid in the determination of the etiologic

relationships of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. EBV-related serology may serve to detect advancing disease, to provide prognostic information, and to monitor the effectiveness of therapy.

Proposed Course: Continue improvement of existing assays (particularly EBNA) and the development of new assays for the measurement of humoral and cell-mediated immunity to EBV-associated antigens. Conduct continued longitudinal studies on African BL patients to provide further evidence for the prognostic significance of EBV-related antibody patterns and to determine, especially, the relationship of anti-EBNA titers to clinical events. Complete studies on NPC in collaboration with Dr. John Ho in Hong Kong. Determine the relationship of EBV titers and T cell function in studies on renal transplantations and infectious mononucleosis.

Date Contract Initiated: March 1, 1973. This is a continuation of the contract PH 43-66-477 initiated February 2, 1966.

Current Annual Level: $169,115

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP4-3395)

Title: Immunological Studies on the Relationship of Embryonic Antigens and Virus-Associated Antigens

Contractor's Project Director: Dr. Samuel A. Wells

Project Officers (NCI): Dr. David Klein
Dr. Robert Friedman (Assistant)

Objectives: To evaluate the immunological similarity known to exist between fetal cell antigens and the antigens of tumor cells induced by oncogenic and endogenous viruses with emphasis on studies on immunity to Schmidt-Ruppin (SR) virus-induced sarcomas in mice.

Major Findings: During the past year a concentrated effort was begun to fully characterize antisera which were produced in the previous years of the contract by immunization of C57Bl mice with (a) syngeneic and allogeneic fetal cells, with and without adjuvant; (b) allogeneic adult spleen cells; (c) Schmidt-Ruppin-D virus-induced carcinomas of C57Bl mice. Serum was also obtained from multiparous C57Bl mice and age-matched virgins, as well as normal controls. These studies were undertaken following the observation that fetal cell immunization was frequently able to protect against transplant challenge by syngeneic SR virus-induced SRD-1 sarcoma cells, and always induced in vitro humoral cytotoxicity against SRD-1 target cells. Transplant resistance and cytotoxic lysis were both related to the virus content of the target cell lines used. It was felt that the relationship between virus content, fetal immunity and tumor immunity could be elucidated using this system.

The procedures for characterizing these antisera included (a) in vitro cytotoxicity using a modified ^{14}C-nicotinamide release assay, testing before and after various absorptions with normal, fetal and tumor cells, and

absorptions with mammalian and avian type C virus; (b) direct and indirect radioimmunoassay against avian and mammalian type C viral proteins and glycoproteins; and (c) ferritin-labeled immuno-electron microscopy.

Due to the small volumes and titers of some of the antisera, the ^{14}C-nicotinamide release assay (Bolognesi, et al., J. Virology 16: 1453, 1975) was modified for use with SRD-1 target cells.

Ferritin immuno-electron microscopy data indicated that (a) anti-fetal antisera can label the surface of the SRD-1 target sarcoma cells and (b) in agreement with previous data, it is the IgM component of these antisera which is active. An interesting observation, which is being pursued, was that labeling frequently took place in the vicinity of virus-like particles in and budding from the target cells.

Significance to Biomedical Research and the Program of the Institute: The hypothesis that depressed host genes are responsible for oncogenesis is being actively studied by several groups in cancer-related programs. The relationship between fetal antigens and virus-associated antigens, particularly those associated with endogenous virus expression, is an important issue in relation to the question of etiology as well as to programs concerned with prevention and control of virus-associated cancers. To understand better the mechanism of neoplastic transformation, it is essential to develop the tools to distinguish between host gene re-expression and new viral gene products.

Proposed Course: This contract will terminate November 30, 1977.

Date Contract Initiated: June 28, 1974

Current Annual Level: $71,500

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CP5-3570)

Title: Demonstration of Tumor-specific Transplantation Antigens in Animal and Human Tumors with the Microcytotoxicity Assay

Contractor's Project Directors: Dr. Karl Erik Hellstrom
Dr. Ingegerd Hellstrom

Project Officer (NCI): Dr. David Klein

Objectives: (1) To continue studies on immune responses against viral and putative embryonic antigens in chemically-induced tumors, using *in vivo* and *in vitro* assay techniques. (2) To develop methods of immunological prevention and of monitoring the development of chemical tumors. (3) To study 'natural immunity' against chemical and virus induced tumors. (4) To study the nature of antigen-antibody complexes in the kidneys of multiparous mice. (5) To study methods of *in vitro* sensitization of lymphocytes against viral proteins. (6) To continue studies on the cellular and humoral immune responses of human subjects against tumor-associated antigens, including those of healthy individuals, tumor patients, and in relatives and close

contacts of tumor patients. In particular, to investigate "natural immunity" to tumors in man.

Major Findings: Embryonic cells and methycholanthrene (MCA)-induced tumor were found to bear MuLV-related surface antigens. Antibodies, probably against these antigens, are found in the sera of mice bearing MCA-induced tumors, and are also found in mice inoculated with MCA; the titers are highest during the mid-latent period and decrease about the time of tumor expression. Using radioimmune assay, a low level of MuLV-related p30 antigen was demonstrated on the surface of primary MCA fibrosarcomas and in spleens of the animals. Higher p30 levels developed when MCA tumors were serially passaged in vivo suggesting replication of infectious MuLV. Antibodies from sera immune to p30 and gp70 bound selectively to cultured MCA tumor cells expressing p30 and MuLV. Mice tended to mount a response to gp70 only if they were bearing tumors that expressed MuLV as well as p30.

Active immunization against embryonic antigens did not delay induction of MCA tumors. Passive immunization, using rabbit anti-mouse embryo serum (unblocking serum) produced significant protection against tumorigenesis and a decrease in blocking activity. Natural antiviral antibody in I-strain mice reacts with MuLV antigens and can sensitize normal lymphocytes against syngeneic MCA tumor cells. The antibody eluted from antigen-antibody complexes found in the kidney glomeruli of multiparious mice had activity against MuLV p12 and p15 proteins. The in vitro sensitization of lymphocytes by means of macrophage-tumor antigen preparations was investigated. Some evidence was obtained that such sensitized lymphocytes are effective in in vivo Winn Tests.

Studies were continued on humoral (membrane immunofluorescence and IPA) immunity to melanoma antigens in patients, their relatives and contacts. Considerable reactivity was observed using sera from different melanoma patients against a number of melanoma cell lines. These sera reacted little with cells from other tumors, and sera from normal controls and other tumor patients reacted less frequently with melanoma cell lines. Preliminary studies confirm that healthy members of two of five families, which each had two family members with melanoma, possessed elevated reactivity against melanoma cells, which could be removed by appropriate absorption. Similar familial humoral studies were begun on human breast carcinoma, while cell-mediated immunity studies were continued on melanoma, breast and lung carcinoma and sarcomas in humans.

Significance to Biomedical Research and the Program of the Institute: Host immune functions are major factors in the control of disease processes, including overt neoplasia. This project was initiated to obtain increased understanding of the immune system in relation to susceptibility and resistance to cancer.

Proposed Course: Studies will be continued on the expression of endogenous MuLV antigens and virus during chemical carcinogenesis, on natural and induced immune responses against endogenous viral antigens, and on in vitro sensitization in cell-mediated immunity.

Since the clinical studies have other sources of funding, clinical studies under this contract will be discontinued.

Date Contract Initiated: April 14, 1969

Current Funding Level: $163,253

GEORGE WASHINGTON UNIVERSITY (NO1-CP2-3251)

Title: In Vivo and In Vitro Studies of the Immune Response to Virus-Associated Antigens in Lymphoma and Breast Cancer Patients and Controls

Contractor's Project Director: Dr. T. Crandall Alford

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To evaluate the immune response of patients with malignant diseases to tumor and virus-associated antigens by means of in vitro assays and skin tests; and to correlate the results with the stage and course of the patient's malignant disease.

Major Findings: Studies on Breast Cancer. Immunologic studies concentrated on the in vitro leukocyte migration inhibition assay (LMI). Patient's leukocytes were exposed to 3M KCl extracts of cells of the breast cancer tissue culture line MCF-7. Breast cancer patients were studied pre-operatively one month after surgery, then at 3 month intervals during follow-up. Controls consisted of gynecological patients with benign breast lesions and other benign and malignant diseases. Testing was done at Litton Bionetics, Inc. on blind specimens collected and coded at George Washington University.

Positive reaction to antigens extracted with 3M KCl from the MCF-7 human breast cancer cell line was observed in the LMI assay with leukocytes from breast cancer patients and from some women with benign breast lesions. Leukocytes from patients with other types of solid tumors and other benign breast diseases generally were not reactive. Leukocytes from some breast cancer patients were positive in the LMI assay when tested against RIII mouse mammary tumor virus or its gp52 antigens; no tests with leukocytes from patients with benign or other cancers were positive. Preliminary evidence suggests that studies of patient general immune competence, as demonstrated by 29 C rosette assays, may provide a useful method of detecting preclinical, recurrent breast cancer.

EBV Studies. Epstein-Barr virus (EBV) is the etiologic agent for infectious mononucleosis (IM), is oncogenic in animals, and has been associated with human Burkitt's Lymphoma (BL) and nasopharyngeal carcinoma (NPC), as demonstrated by high antibody titers to EBV and the presence of viral genome in tumor cells. The cellular response to EBV antigens has been less well studied. Attempts were made to correlate in vitro cellular (LMI) responses to antigen preparations from Raji cells, with in vivo skin test responses. In 13 patients, all but 2 responded to skin test recall antigens (candida or diphtheria-tetanus). One antigen preparation (HMP 25-3340-29) elicited a positive response in 7/13

patients; the other preparations were less antigenic. Using Raji HMP 25-3340-29 as antigen in LMI tests, very little correlation was observed with the skin test results. In contrast, high correlation was observed between skin test and LMI in PPD (tuberculin)-positive and negative individuals.

Significance to Biomedical Research and the Program of the Institute: Correlations between clinical course of cancer and LMI to antigens, such as MCF-7 extract or MMTV antigens, are of value for prognostic and diagnostic applications.

Proposed Course: This contract was terminated January 12, 1977

Date Contract Initiated: April 13, 1972

Current Annual Level: $64,500

HEBREW UNIVERSITY-HADASSAH MEDICAL SCHOOL, JERUSALEM (NO1-CP3-3342)

Title: A Multidisciplinary Study of Hodgkin's Disease in Israel

Contractor's Project Director: Dr. Natan Goldblum

Project Officer (NCI): Dr. Paul Levine

Objectives: To investigate the interrelationship between environmental and genetic factors in the etiology, pathogenesis and spread of Hodgkin's disease (HD) in Israel and, in particular: (1) to evaluate clustering among social contacts, in space and time, and to develop methods for the study of clustering; (2) to evaluate HD in immigrant and native-born Israelis, including studies of HL-A typing, and antibody levels to Epstein-Barr virus (EBV) and other viruses; (3) to review the histological sections of all malignant lymphomas diagnosed throughout Israel during 1964-1972 so as to provide basic information necessary for the immuno-epidemiologic studies; and (4) to establish laboratory assays relevant to an understanding of the pathogenesis of malignant lymphoma.

Major Findings: An analysis of the incidence of Hodgkin's disease (HD) and its various histologic subtypes between the years 1960-1972 has been completed. Data on the survival of HD patients diagnosed during these years have been coded and analysis will commence shortly. Data on 499 case-control pairs are now available for analysis of "clustering in space and time" and among social contacts. The distribution of 28 HL-A antigens was studied in 137 Israeli patients with HD. A high frequency of the second sublocus, W35, was established. Since a high incidence of W35 is not infrequent in certain population groups examined, the validity of these findings must be verified by studies of matched controls and various population groups. Such studies were carried out and include: 127 individuals originating from Poland, 123 from Russia, 130 from Romania, 141 from Morocco, 123 from Iraq, 180 Christian Arabs, and 120 Druze. The results are being summarized.

Further studies were carried out to establish the diagnostic usefulness of the fluorescent concanavalin A probe as a differential diagnostic test for HD and other types of lymphoma. Tests of blood samples from over 300 patients with HD, other lymphomas and chronic lymphocytic leukemia have been completed; they were compared with 115 healthy individuals and over 700 patients with other disorders. The results indicate, unequivocally, that this probe can be used effectively in clinical practice as an important aid in the diagnosis of HD and other lymphomas. Studies have also been made on the differences in the degree of membrane fluidity between lymphocytes from HD and other lymphoma patients and from normal individuals using 1,6 diphenyl 1,3,5 hexatriene (DPH) as a probe. Lymphocytes from patients with HD and lymphoma have a more fluid lipid layer than lymphocytes from normal individuals. Studies on lymphoblastoid cells in culture clearly indicate the presence in Israel, side by side, of two types of Burkitt's lymphoma: the EBV-genome carrying (African) type and the EBV-negative Burkitt's tumor (North European or American Burkitt). Approximately 63 lymphoblastoid cell lines were established from patients with HD, other malignant lymphomas, other malignancies and reactive lymphadenopathies. The majority of these lines were typical of those described by others. One line, derived from a child with a "Burkitt-like" tumor, is highly atypical. Its morphology resembles transitional bone marrow cells; it lacks EBV genome, EBV receptors, or a high cap-forming ability with fluorescent con A. It forms neither EAC nor E rosettes, but seems to be B-cell in origin, as demonstrated by IgM-Kappa immunoglobulins on the surface. It possesses an apparent atypical karyotype similar to that described in tumor biopsies anc cultures derived from patients with African Burkitt's lymphoma.

Significance to Biomedical Research and the Program of the Institute: This project permits a comprehensive study of all lymphoma cases arising in a large, well-defined population, with comparable measurement of virus-exposure, immunologic status, and genetic background on a control group. Mortality data, an unrealiable but useful indicator of environmental contributors to cancer etiology, have shown that the country of origin plays an important role in determining which type of tumor will develop in individuals migrating to Israel. The project is now emphasizing incidence studies, and it may be possible to determine at what age and in what country environmental carcinogens exert their influence in determining the future development of cancer. Although genetics appear to play a role in the incidence of HD, there is also evidence that an oncogenic virus is involved.

Proposed Course: This contract terminated on July 28, 1977.

Date Contract Initiated: June 29, 1973

Current Annual Level: $150,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP4-3296)

Title: Sero-Epidemiologic and Laboratory Studies on Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de The

Project Officer (NCI): Dr. Robert Goldberg
Dr. Paul H. Levine (Assistant)

Objectives: To investigate the Epstein-Barr virus (EBV) in relation to Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) in order to determine its role in the etiology of these neoplasms.

The first specific objective is to conduct a 5 year prospective seroepidemiological study in a child cohort population of 35,000 in the West Nile District of Uganda to test the validity of four hypotheses regarding the etiological relationship between EBV and BL. Two sub-objectives are: (1) to study the role of chronic malaria in the development of BL; and (2) to investigate the epidemiological behavior of EBV infection in the study population by follow-up of randomly selected groups.

The second specific objective is an investigation of the sero-epidemiological behavior of EBV in populations at different risk for EBV-associated diseases, carried out in Hong Kong, Singapore, Uganda, and Tunisia.

The third objective is the investigation of the possible role of EBV in the development of NPC through the integration of field and laboratory studies.

Major Findings: Burkitt's Lymphoma (BL) Program: Twelve BL cases were detected in the 42,000 children bled in the main collection. Ten of these were tested in parallel by Dr. W. Henle and IARC laboratories. Comparison between the "pre-BL" sera and controls at the same time as the main collection and population samples bled in the sub-study) showed that virus capsid antigen (VCA) titers of pre-BL sera was significantly higher than that of both controls in six cases out of ten; two cases were in the same range and two were lower. DNA hybridization tests showed that two tumors, one in the average group and one with a very low EBV profile, gave results below the sensitivity of the of the assay, using the COT technique. Other reactivities in the pre-BL sera did not differ significantly from the control. At the time of BL development, antibodies to early antigen appeared in five cases and increased in two other cases. Such preliminary results suggest that: (a) VCA antibodies do not protect against BL occurrence; (b) high VCA titers represent a risk factor, possibly linked with very heavy EBV infection early in life; (c) onset of BL is accompanied by a reactivation of a chronic EBV infection. Another ten to fifteen cases would be needed before more precise conclusions could be reached.

BL case detection in the Mara districts of Tanzania showed a BL incidence similar to that of the West Nile. A malaria intervention project by chloroquine treatment in children in this area is being implemented. This provides opportunity to determine whether the incidence of BL will decrease following malaria control.

Sero-Epidemiology of EBV: In Uganda 20% of the children have EA antibodies by the age of 1-4 years, followed by a progressive decline of such antibodies

throughout the rest of their lives. The Chinese by contrast show a later rise in EA antibodies, but in a much higher percentage of individuals.

Nasopharyngeal Carcinoma (NPC) Program: Immunogenetic studies confirmed the HLA haplotype A-Sin 2 and B-Sin 2 in Chinese as a marker for NPC risk at the A and B loci. Identification of a marker at the D locus (Sin 2a) is in progress. In vivo cell mediated immunity studies confirmed high skin reactivity of NPC patients to membrane extracts of an NPC-derived, EBV-producing cell line (HKLY-28) as compared to that of patients with other tumors; in vitro studies indicated that both lymphocyte activation and migration inhibitory factor (MIF) release assays using membrane extracts prepared from Raji cells and NPC biopsies allow a discrimination between NPC patients and other tumor patients.

Significance to Biomedical Research and the Program of the Institute: To date, EBV is the only naturally-occurring virus strongly suspected of an etiologic role in human cancer. The research under this contract should help elucidate the role of EBV in two human tumors, Burkitt's lymphoma and nasopharyngeal carcinoma. Initial tests on individuals of various ethnic groups shows that there are significant differences among populations at different risk of NPC. The results obtained by blood genetic typing, together with the seroepidemiological results, may provide the means for detecting high risk groups among a normal population for NPC.

Proposed Course: In Africa, case detection will continue to have a high priority in order to determine the relationship of EBV antibodies to subsequent appearance of disease. The nasopharyngeal carcinoma program will be continued to define the genetic factors relating to disease. Immunologic and virologic studies will be conducted to determine whether or not the EBV isolated from Burkitt lymphoma cases is identical to that isolated from NPC.

Date Contract Initiated: June 11, 1970

Current Annual Level: $700,000

LITTON BIONETICS, INC. (NO1-CP4-3333)

Title: Immunological Assays for DNA and RNA Viruses

Contractor's Project Director: Dr. David Fuccillo

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To analyze humoral and cellular immune functions in normal individuals and cancer patients against Epstein-Barr virus (EBV) and to apply immunologic assays measuring EBV immunity to the control of human cancers, primarily Burkitt's lymphoma and nasopharyngeal carcinoma. To support other investigators in the cancer field by means of expertise in assays and/or distribution of materials and reagents collected and stored as a result of the contract.

Major Findings: The contractor standardized the immunologic assays important to the EBV clinical studies. Four assays were used: leukocyte adherence inhibition (LAI); antibody-dependent lymphocyte cytotoxicity (ADLC); lymphocyte proliferation; and leukocyte migration inhibition.

The LAI assay was used to test for cell-mediated immunity (CMI) to EBV antigens in 22 patients with infectious mononucleosis (IM), 47 lymphoma patients, 101 carcinoma patients, and 84 patients without cancer. Response to EB virion ("v") antigen was generally present at the time of diagnosis in the IM patients, but the response to EBV soluble ("S") antigen was delayed. An increased CMI response to "v" antigen was found in patients with IM, Hodgkin's disease and non-Hodgkin's disease had depressed response to the EBV-associated "S" antigen. The finding of increased LAI response to "v" antigen in Hodgkin's disease patients with high EBV antibody titers conflicts with previous reports that attribute high antibody responses to a generalized depressed cell-mediated immunity.

The study on nasopharyngeal carcinoma (NPC) patients was accomplished as an international study which included patients from the U.S., France, Tunisia and the Far East and a domestic study with patients from the U.S. only. The antibody response of NPC patients to EBV viral capsid antigens (VCA), early antigens (EA), soluble complement-fixing antigens (CF/S), EBV nuclear antigens (EBNA) and to membrane antigens by ADLC was determined. In NPC patients in the international study, antibody titers to VCA and EA were higher than in controls, while titers against EBNA, CF/S and ADLC were only slightly elevated compared to controls. In the domestic study, antibody titers to all these virus-specific antigens, except EBNA, were significantly higher in NPC patients than in matched controls.

Human tumor tissue samples received for frozen storage in a viable state were first characterized for HL-A type and examined for sterility, the presence of mycoplasma, cytopathic viruses, mammalian type C viral reverse transcriptase, EBV antigens and immunological surface receptors. Similar determinations were made on antigen preparations from tumor samples supplied to different groups of investigators. Multiple samples of well-documented skin biopsies and other cancer case materials were sent to different investigators.

Significance to Biomedical Research and the Program of the Institute: Epstein-Barr virus is oncogenic in some monkey species, is ubiquitous in the human population, and is known to cause at least two clinical syndromes, infectious mononucleosis and abacterial tonsillitis. There is increasing evidence that it is involved in the cause of Burkitt's lymphoma, nasopharyngeal carcinoma, and Guillaine-Barre, an infectious neurologic disease. It is of great importance to acquire information on the different factors that may influence the outcome of EBV infection, particularly development of cancer.

Proposed Course: Studies will continue to focus on defining the disease-related patterns of immune responses to EBV in humans.

Date Contract Initiated: June 3, 1974

Current Annual Level: $443,946

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Directors: Dr. Paul H. Black
Dr. Martin S. Hirsch

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: To determine the effects of human interferon in the prophylaxis of virus infections in immunosupressed kidney transplant patients. To study the cellular reactivity of normal humans and of patients with neoplastic and other disorders against primate oncornavirus antigens.

Major Findings: Studies of cell-mediated immune responses of mice against antigens associated with endogenous type C oncornaviruses were completed. Further studies in the mouse model on inhibition of activation of endogenous type C viruses by interferon were discontinued.

A major effort has been a double-blind, placebo-controlled study, commencing 2/14/76, of human leukocyte interferon prophylaxis against virus infection and cancer in renal transplant patients. Twelve patients have been entered into the study. The initial therapy, consisting of 3,000,000 units of interferon every 2 days for 4 weeks following transplantation, was amended following reversible toxicity effects in 2 patients (leukopenia and thrombocytopenia). It was found that interferon was not removed during hemodialysis, and the toxicity was attributed to interferon retention, due to renal impairment. With the same dose of interferon given twice weekly for 6 weeks, no further toxicity symptoms were observed.

Of 9 patients followed for a period of 6 months, 7 excreted herpes simplex virus (HSV) detected in throat washings for varying periods; three shed cytomegalovirus (CMV) intermittently in urine and 2 in throat secretions. Two patients had clinically severe HSV stomatitis; one had a febrile leukopenia associated with CMV viremia; and one patient developed warts. Seven kidney rejection episodes occurred. Any clear correlation between these clinical observations and the interferon therapy awaits the breaking of the double-blind code.

Cell-mediated immunity (CMI) against simian sarcoma virus (SSV) -associated antigens was demonstrated in 3 of 14 human volunteers by microcytotoxicity tests and in 1 of 3 volunteers by lymphocyte proliferation assay. Studies on the nature of the lymphocytes involved (T, B, K, null) are in progress. Similar studies are in progress using leukocytes from patients with disorders such as Hodgkin's disease, chronic lymphocytic leukemia, acute myelocytic leukemia and systemic lupus erythematosus.

A logitudinal study of CMI against both SSV and M-7 baboon virus is being carried out in healthy normal women during pregnancy and following parturition.

Significance to Biomedical Research and the Program of the Institute: Although cancer risk in organ transplant patients is high, it is unlikely with the small number of patients receiving interferon that any significant differences in the incidence of cancer will be detected. However, the studies on the inhibition of virus infection in these immunosuppressed patients should provide valuable data on interferon prophylaxis, which may later be more directly applicable to cancer prophylaxis and therapy. The information obtained on human cellular reactivity to simian sarcoma viruses is pertinent in the context of other Program investigations on viral relationships to human cancers.

Proposed Course: The current clinical studies will be continued according to the protocols already established, with appropriate modifications, as these seem clinically indicated.

Date Contract Initiated: September 15, 1971

Current Annual Level: $168,344

MERCK AND COMPANY, INC. (NO1-CP1-2059)

Title: Research on Oncogenic and Potentially Oncogenic Viruses, Virus Production and Vaccine Development

Contractor's Project Directors: Dr. Maurice Hilleman
Dr. Vivian Larson

Project Officer (NCI): Dr. Michael Chirigos

Objectives: The overall objective is to determine the practical feasibility for immunologic prevention of virus-induced cancer applicable for use in humans. The specific objectives are: (1) Project A - to develop a Herpes simplex type 2 (HSV2) subunit vaccine suitable for prophylactic trials in man against genital herpetic disease and against possible subsequent herpesvirus-induced cancer; and (2) Project B - to evolve type C virus vaccine technology for future application in preparation of leukemia virus vaccines suitable for use in humans by developing a safe and effective vaccine against leukemia in the cat model system.

Major Findings: Both vaccine development programs were brought to the stage of preparation of experimental virus glycoprotein vaccine production and evaluation in animals.

Project A: Approximately 4000 mg of crude HSV-2 antigen was prepared and stored at -70C. Purification of the antigen by preparative poly-acrylamide gel electrophoresis (PAGE) using SDS/urea solubilization was discontinued after about 3.4 mg of purified gp126 antigen was obtained. The current technique uses Triton X-100, 0.5M NaCl solubilization, with subsequent chromatography on DEAE cellulose.

Potency tests of HSV-2 vaccines in guinea pigs, based on the induction of neutralizing antibodies (plaque reduction assay) and cell-mediated immunity (lymphocyte stimulation) yielded the following results: (1) alum adsorption of a gp126-enriched fraction (PAGE-SDS/urea preparation) significantly enhanced immunity as compared with aqueous and adjuvant 65 vaccines. (2) Triton X-100, 0.5M NaCl solubilization of the crude HSV-2 antigen did not severely reduce immunogenicity as was previously observed with SDS/urea solubilization of the same antigen. (3) Alum was the most effective adjuvant for the crude unfractionated Triton X-100, 0.5M NaCl solubilized antigen, as compared to Freund's incomplete adjuvant, adjuvant 65 or aqueous vaccines. (4) Both the gp126-rich subunit antigen, prepared by sequential Triton X-100 extraction, and the DEAE glycoprotein-rich DNA-free fraction extracted from it were immunogenic at 10 ug or 80 ug dose levels as alum-adsorbed or Freund's complete adjuvant vaccines.

Three hundred immunogenic 10 ug doses of the DEAE virus glycoprotein-rich fraction were obtained from each chick embryo used to produce the crude antigen. The project aims to further purify the vaccine.

Vaccine protective efficiency is also evaluated in guinea pigs by vaginal challenge with live HSV-2 virus. So far only slight protection was observed using adjuvant 65 and alum gp126 vaccines prepared from SDS solubilized antigens. Vaccines prepared from Triton X-100, 0.5M NaCl solubilized antigens are currently under test.

Project B: Feline Leukemia Virus (FeLV) Vaccine. Quantities of FeLV virus (Jarrett's FeLV-1 strain) and the 75,000 dalton viral envelope glycoprotein (gp75) were produced for glycoprotein vaccine studies in animals. Potency tests were carried out in guinea pigs and cats and the immune response to gp75 vaccines was measured by the induction of neutralizing antibody (reverse transcriptase induction assay), cytotoxic antibody (^{51}Cr release assay) and radioimmune precipitating antibody. In guinea pigs, adjuvant 65 significantly enhanced the immunogenicity of gp75 in a 20 ug dose as compared to aqueous vaccine. However, in cats, adjuvant 65 vaccine did not stimulate a significant immune response even at twice the dose level (40 ug) that was immunogenic in guinea pigs. The aqueous vaccine at the same dose level did not seroconvert any cats. No protection against infection was observed when these cats were challenged with live homologous virus. Potency tests are in progress in cats and in guinea pigs with alum-adsorbed gp75 vaccines.

Significance to Biomedical Research and the Program of the Institute: If viruses are proven to cause some human cancers, a vaccine may effectively prevent or minimize infection or pre-sensitize the host to reject antigens expressed during the earliest stages of tumor development. Since live attenuated or killed virus vaccines for potentially oncogenic viruses would not be acceptable for human use due to the danger of transfer of functional viral genetic material, this project was initiated to determine whether vaccines to purified viral antigens acceptable for use in humans were of practical value.

Proposed Course: This project is expected to be completed during the next year.

Date Contract Initiated: March 1, 1971

Current Annual Level: $800,000

MICROBIOLOGICAL ASSOCIATES (NO1-CP3-3248)

Title: Immunoprevention of Spontaneously Occurring Neoplasms

Contractor's Project Director: Dr. Bernard Sass

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: To examine immunoprevention of spontaneously-occurring tumors in laboratory mice by the use of viral and cellular vaccines. This includes studies of the parameters involved in spontaneous neoplasia including incidence, progression and histologic types of tumors, endogenous type C virus expression, and natural and induced humoral and cellular immunity to viral and nonviral tumor antigens.

Major Findings: Efforts were focused on the immunoprevention of spontaneous neoplasms in animal model systems with similarities to human neoplasms. Past efforts defined the ecotropic type C virus and spontaneous tumor expression in large holding colonies of untreated, aging mice chosen to serve as specific model systems. More recently a number of immunoassays were applied to measure the natural and induced host immune responses to type C viruses and virus subunits. Immune responses to type C viruses and type C virus containing tumor cells were measured after immunization with virus and virus containing cellular vaccines. In the past contract year, five major studies were completed. The results showed: (1) A reduction of X-ray induced leukemias in C57Bl mice by induction of active immunity or transfer of passive immunity to endogenous type C virus. Active immunity was established by immunization with inactivated Rauscher murine leukemia virus (R-MuLV) and passive immunity by transfer of goat anti-Gross-MuLV immune IgG. Both regimens resulted in measurable circulating antibody to AKR-gp70 throughout part of the latent period of tumor development, both resulted in reduced tumor incidence compared to several controls, and both resulted in apparent immunoselection against virus positive tumors in the tumors that occurred in these groups. (2) Induction of active immunity in the BALB/c mouse by type C virus vaccines conferred resistance to exogenous virus challenge and suppressed endogenous type C virus in the spleens of these animals. Both Rauscher leukemia virus and Gross leukemia virus vaccines were effective, and both induced cell-mediated immunity and low level neutralizing antibodies. Purified virus gp70 also induced resistance to challenge with Rauscher leukemia virus. (3) Resistance to virus-free syngeneic tumor transplantation in the BALB/c mouse after immunization with type C virus-infected homologous tumor cells. Splenic lymphocytes from the immune animal were shown to be cytotoxic for tumor cells in vitro and, by adoptive transfer, were capable of tumor prevention in vivo. (4) Virus-specific complement dependent cytolytic activity for R-MuLV-infected cells treated with anti-R-gp70 and with anti-R-p12 sera. (5) The ubiquitous presence

of either ecotropic or xenotropic viral antigens in low leukemia incidence BALB/c mice and of xenotropic viral antigens in low leukemia incidence aged NIH Swiss mice. The spontaneous neoplasms of the NIH Swiss mice have the p12 antigen of the xenotropic virus, and monospecific antisera to the p12 antigen showed this antigen to be on the cell surface where it can serve as a target antigen for antibody mediated cell lysis.

Significance to Biomedical Research and the Program of the Institute: This contract contributes to an understanding of the genetic and epigenetic relationship of endogenous type C viruses to the host's naturally occurring tumors and explores immunologic approaches to tumor control.

Proposed Course: Investigate measures for immunoprevention of spontaneously occurring tumors in the BALB/c mouse with viral and cellular vaccines; attempt immunoprevention of chemically induced tumors in BALB/c and C57Bl mice with vaccines produced from cells containing type C virus; and study immunologic factors in C57Bl mice with radiation-induced tumors. Immune response potential will be studied in aging mice in conjunction with endogenous virus expression. The complex interaction of humoral and cellular immunity and their respective roles in spontaneous neoplasia occurring with advanced age will be studied.

Date Contract Initiated: November 15, 1961

Current Annual Level: $705,000

MOUNT SINAI SCHOOL OF MEDICINE AND HOSPITAL (NO1-CP4-3225)

Title: Stimulation of Immunity to Virus-Associated and Tumor-Associated Antigens in Mouse Systems

Contractor's Project Director: Dr. J. George Bekesi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To evaluate chemoimmunotherapeutic measures in tumor-bearing animals using neuraminidase-treated cells and viral inhibitors to determine the best means for controlling the oncogenic virus and the tumor. (2) To examine the virus titer and specific anti-virus immunity in animals undergoing chemoimmunotherapy and in untreated controls. (3) To transfer the knowledge gained from these animal studies to clinical applications against human leukemia.

Major Findings: Leukemia Mouse Model. Immunization of leukemic AKR mice with neuraminidase-treated spontaneous syngeneic leukemic thymocytes, or with allogeneic E_2G leukemic cells after cytoreductive chemotherapy delayed reappearance of primary lymphomas. The median survival time was greater than 160 days following chemoimmunotherapy as compared to 12 days for untreated mice, and 35 days of mice given chemotherapy alone. Similar experiments substituting a broad spectrum anti-viral agent, Virazole, for the antigen treatment also resulted in remission and an increased lifespan. Purified mouse interferon was tested in chronically infected AKR mice, both in the preleukemic stage and after diagnosis of disease. Viral titers decreased and remained low for as long

as 120 days. Prophylactic interferon therapy caused a significant delay in the appearance of primary spontaneous leukemia and, when used against established disease, resulted in reduced spleen weights, MuLV titers and a statistically significant increase in lifespan ($p=0.001$). The sera of nonleukemic AKR mice were found to contain two types of autoantibodies, both IgM. One of these reacted preferentially with AKR thymus cells, and was considered possibly to contribute to the leukemogenic process by attachment to thymus cells and rendering them more susceptible to the leukemogenic effects of the virus. The second type of antibodies reacted more readily with neuraminidase-treated leukemic cells. It was postulated that these antibodies may play a role in the immunoreactivity of AKR mice against leukemia antigens, and may be involved in the immunotherapeutic effects of neuraminidase-treated leukemic cells.

Clinical Leukemia Studies. In patients with acute monocytic leukemia under ara-C/daunorubicin-induced complete clinical remission, administration of 2×10^8 neuraminidase-treated allogeneic myeloblasts following each chemotherapy cycle produced a highly significant prolongation in the duration of remission (20/28 in remission, 12 of the 20 for over 100 weeks as compared to a 26 week median remission for chemotherapy alone). Immunized patients were observed to have greater *in vivo* and *in vitro* immunocompetence.

Mouse Mammary Tumor Model. In C3H mice, total excision of the first spontaneous mammary tumor followed by immunization with neuraminidase-treated allogeneic tumor cells significantly delayed the appearance of a second primary carcinoma at another mammary gland site and prolonged life span. Neuraminidase-treated syngeneic and autochthonous tumor cells were less effective as immunogens.

Interferon administration following total excision of the primary tumor also significantly delayed or completely prevented appearance of the second primary tumor. Soluble antigens from mouse mammary tumor virus (MMTV)-induced mammary tumors stimulated *in vitro* DNA synthesis in spleen cells from mice bearing MMTV-induced mammary tumors. This activity was inhibited by serum from tumor-bearing animals.

Herpesvirus Saimiri (HVS)-Induced Primate Lymphoma Model. Studies were carried out on the possibility of antiviral therapy against HVS-induced malignant lymphomas in owl monkeys. Phosphonoacetic acid (PAA) completely inhibited HVS replication in tissue culture and the conditions for its action were defined. Preclinical toxicity trials demonstrated that PAA in potentially prophylactic doses is well tolerated. Prophylactic trials against HVS infection *in vivo* are planned.

Significance to Biomedical Research and the Program of the Institute: Evidence suggests that in acute leukemia, breast cancer, and Burkitt's lymphoma, late relapse may actually be disease reinduction due to persistence of the factors initially associated with the disease. If there is a viral etiology for these three tumors, it is important to obtain methods of permanently controlling the virus while chemotherapy or immunotherapy are used to control the tumor itself. Successful development of treatment protocols in animals with leukemia, breast cancer, and lymphoma are an important first step in curing these forms of human cancer.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: August 6, 1973

Current Annual Level: $290,000

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP5-3571)

Title: Immunobiologic Response of the Cat to Feline Oncornavirus

Contractor's Project Director: Dr. Richard G. Olsen

Project Officer (NCI): Dr. Alfred Hellaman

Objectives: Studies concentrated on development of a regimen for inducing immunity to feline oncornavirus and virus-specified tumor antigens. Specific objectives are: (1) To solubilize and characterize the feline oncornavirus-associated cell membrane antigen (FOCMA); (2) to prepare purified feline leukemia virus (FeLV) envelope glycoproteins (gp 69/71); (3) to evaluate the vaccine potential of FOCMA and FeLV gp 69/71 for the prevention of viremia and tumorigenesis in specific pathogen free cats; (4) to evaluate the lymphocyte blast transformation responses to defined FOCMA and FeLV gp 69/71 antigens in vaccinated cats and cats infected with virulent feline oncornaviruses; (5) to determine whether a chemical carcinogen, methylnitrosourea, alters the age-related susceptibility of cats to feline oncornavirus disease; (6) to determine whether chemical carcinogens alter the immunobiologic responses of cats to feline oncornaviruses; (7) to investigate further the relationship of age, virus strain, route of infection, and other immunologic or cocarcinogenic factors which influence the susceptibility of cats to feline oncornaviruses; (8) to evaluate the mixed lymphocyte-tumor cell assay using sensitized feline peripheral blood lymphocytes and inactivated lymphoblastoid cells to detect tumor-associated antigen blast transformation.

Major Findings: During the past year a feline oncornavirus vaccine was developed and evaluated. Immunization of kittens with virus-inactivated (FOCMA containing) FL 74 feline lymphoblastoid cells induced specific FOCMA antibody. These cats were resistant to malignant tumor development after challenge with oncogenic doses of feline oncornavirus. FOCMA vaccine, however, did not prevent FeLV viremia.

In studies to prevent FeLV viremia and malignant tumor growth, kittens were actively immunized with a combined vaccine composed of UV-inactiviated FeLV and killed FL-74 cells. After oncogenic challenge, kittens that had been given a combined FeLV/FL-74 vaccine developed lower FOCMA antibody titers than kittens immunized with FL-74 alone. In additon, kittens given the combined FeLV/FL-74 vaccine appeared to demonstrate tumor enhancement after challenge with oncogenic virus. Inactivated FeLV also failed to induce protective immunity in kittens suckling on dams that had been given multiple immunizations during gestation.

Since killed virus appeared to cause immunosuppression in young kittens, studies were initiated to evaluate the influence on feline T-lymphocyte function of killed FeLV and FeLV subviral components, p15, p27, and gp69/71. Killed FeLV and FeLV p15 inhibited blast transformation responses of cat lymphocytes to con A, but FeLV p27, FeLV gp69/71 and RD-114 virus did not.

FOCMA antigen has been solubilized by papain digestion from FL-74 cells. However, higher yields of FOCMA have been obtained from supernatant fluids from FL-74 cells maintained in serum-free medium. Soluble FOCMA is being purified and is being evaluated for vaccine potential.

Investigations into the pathogenesis of FeLV infection showed that susceptibility to FeLV and to feline sarcoma virus FeSV was age-related (4 strains tested) and, therefore, had to be considered in designing viral challenge experiments. Ancillary studies demonstrated that the presence of FeLV group specific antigen (gsa) in the blood cells of cats correlated with infectious virus in blood plasma. The viral infectivity and gsa tests were of comparable sensitivity in detecting the onset of FeLV viremia.

Preliminary studies showed treatment of cats with methylnitrosourea increased the susceptibility of cats to FeLV infection.

Significance to Biomedical Research and the Program of the Institute: Prophylactic immunization protocols in model systems, such as those carried out under this contract, evaluate prospects for control of cancer induced by horizontally transmitted viruses. The information obtained in such work may be applicable to prophylaxis against other animal oncornaviruses and may possibly have particular future value in control of some human neoplastic diseases.

Proposed Course: A concentrated effort will be made to isolate and purify FOCMA soluble vaccines and FeLV proteins, including gp69/71 and p15. As these become available, vaccination procedures will be established with regard to host age, virus strain, immunization route and dose. Mixed vaccines of soluble FOCMA-FeLV envelope proteins will continue to be investigated. The biological responses to live and killed virus and viral proteins will be studied from the point of view of: (a) the primary site for expression of FOCMA; (b) the etiology of immunosuppression by FeLV proteins, including the production of serum factors, interfering antibodies, etc.; (c) the transmission and excretion of virus in gsa-positive and -negative cats; macrophage function and other host defense components in resistance to feline oncornaviruses, as determined by mixed lymphocyte-tumor interaction, antibody-dependent cellular cytotoxicity, blocking antibody and suppressor cell formation.

Date Contract Initiated: June 25, 1965

Current Annual Level: $220,823

RUSH-PRESBYTERIAN-ST LUKE'S MEDICAL CENTER (NO1-CP3-3219)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officer (NCI): Dr. Raoul Benveniste

Objectives: (1) To maintain a marmoset breeding colony. (2) To study responses of these primates to viruses known or suspected to produce cancers in humans or in animals.

Major Findings: The breeding of marmosets was continued. During the past year, a marmoset breeding colony, previously maintained at the Lincoln Park Zoo, was transferred to this Center. Among 225 male/female pairs there were 97 pregnancies with 135 live births. Accurate RIA and HAI tests for pregnancy in marmosets were developed.

The effects of the B95-8 strain of Epstein Barr Virus (EBV) in marmoset subspecies ranged from no discernable disease, through self-limiting mononucleosis to progressive lymphomas. Abundant EBV-DNA was produced in Raji cells. Using degredative enzymology and molecular hybridization, a cell clone was identified containing only one EBV genome/cell. Herpesvirus papio (HVP), an EBV-like herpes agent, was isolated from baboon lymphoblastoid cell lines. Studies on the pathogenicity of HVP in marmosets and an analysis of HVP-transformed cells were begun.

It has been shown that primate lymphoblastoid cell lines may be established by infection in vitro with Herpesvirus saimiri (HVS) or Herpesvirus ateles (HVA). Though it was possible to transform rat embryo cells with Herpes Simplex virus type 2 (HSV-2), attempts to transform marmoset cells have not succeeded. Infection with attenuated HVS protected marmosets against challenge by oncogenic HVS. Marmoset cells infected with simian sarcoma virus (SSV-1) and SSV-1-associated virus (SSAV-1) were further characterized. A variant SSV-1/SSAV-1 with altered antigenicity was demonstrated. This was considered possibly to be a recombinant with an endogenous marmoset virus. An SSV-1 marmoset glioma cell model was established by intracerebral inoculation of SSV-1/SSAV-1.

Studies were undertaken on human sarcomas, lymphomas and leukemias in an attempt to isolate oncornaviruses. None have been detected.

Significance to Biomedical Research and the Program of the Institute: Restrictions in the host range of viruses require the availability of a primate animal for the study of viruses associated with neoplasia in humans. The marmoset has proven to be most responsive to the oncogenic activity of known tumor viruses. This species is particularly valuable for the study of herpesvirus strains that induce malignant transformation. Viruses of the herpes group are widely distributed among animal species, and infections by a number of these agents are known to result in the development of malignant tumors in the host. It is most important to expand our knowledge of herpesviruses with respect to cancer in primate animals and of their potential for oncogenicity in humans.

Proposed Course: This contract will be terminated on September 30, 1977

Date Contract Initiated: March 15, 1962

Current Annual Level: $325,000

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF, AND CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP5-3500)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Brian E. Henderson (USC)
Dr. Robert M. McAllister (Children's Hosp.)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at the Children's Hospital of Los Angeles.

Viral Studies: Human, animal pet and fetal animal cancer and fetal materials are under intensive study for RNA tumor virus genetic expression, using all the modern in vitro as well as in vivo test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials are used for in vitro and in vivo biological studies and are subjected to serological, immunological, biochemical and electron microscopic analyses designed to detect, isolate and characterize RNA viruses and virus-specific antigens associated with naturally occurring animal and human neoplasms.

Epidemiological Studies: This program is providing, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer as it may be influenced by genetic, viral, environmental or other factors, including exposure to variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants are being studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental Studies: This program is concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected are characterized and supplied to laboratories at USC as well as to NCI and other VCP contract programs; e.g., Contract NO1-CP4-3240 for studies

to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Major Findings: In lymphoma-prone high virus expressor LC wild mice, tumors occur in a familial pattern; indigenous murine leukemia virus (MuLV) is transmitted as a maternal congenital infection with immune tolerance and persistent life-long infection. Lymphomas in these mice arise in bone marrow and B cell areas of the spleen and are reduced in incidence by splenectomy but not by thymectomy. In crosses between LC and susceptible Fv-1^{nn} laboratory mice, the wild mouse virus is transmitted to F1 progeny, probably exclusively as a ongenital infection.

A new class of "amphotropic" virus in wild mice is probably the parental virus from which the standard ecotropic and xenotropic viruses of laboratory mice were derived. The amphotropic viruses are lymphomagenic but not neurotropic. Wild mice also harbor an ecotropic class of MuLV which is related to but distinct from AKR mouse virus and entirely unrelated to the FMR mouse virus group. The ecotropic viruses are both lymphomagenic and neurotropic. Amphotropic virus is more prevalent and exists in higher titers than ecotropic virus in most wild mice.

Indigenous leukemia virus in the lymphoma prone LC wild mice is largely suppressible by passive immunization of newborns with specific viral antisera.

Rat embryo cells shedding endogenous type C virus undergo accelerated transformation which can be further hastened by addition of chemical carcinogens. Type C virus was isolated from a wild rat (Rattus rattus).

The horizontal spread of feline leukemia virus (FeLV) among household cats is confirmed. Humans in contact with FeLV positive cats show no immunologic evidence of FeLV infection. Feline orcornavirus cell membrane antigen (FOCMA) is probably a tumor specific antigen in cats; its expression is not soley dependent upon exogenous infection with FeLV. Noninfectious expression of RD-114 virus RNA, p30, and gp70 is found in all natural cat lymphomas and in most naturally occurring cat sarcomas and carcinomas. Expression of FeLV RNA, p30, and gp70 generally indicates systemic infection with FeLV and is found in the vast majority of lymphomas in young cats but only in a small minority of lymphomas, sarcomas, and carcinomas in older cats.

The p12 phosphoprotein of mammalian type C viruses binds specifically to the homologous viral RNA and the phosphorylated state of this protein may be involved in regulation of viral reverse transcriptase activity.

An extensive search for human type C virus was negative and no evidence of infection of humans with primate-derived type C viruses was found.

An Epstein Barr virus (EBV)-like herpes virus was isolated from a hematogenous cell line derived from an orangutan with spontaneous myelomonocytic leukemia.

Homologous regions, possibly representing pol and gag genes of the baboon and RD-114 viruses were mapped by electron microscopic observations on heteroduplex formation. Electron microscopy of the physical nature of mammalian

type C viral RNA genomes indicates a dimer structure containing two monomer subunits 8-10 kilobases in length (molecular weight 3 x 10^6 daltons). Transfection with RD-114 virus using cleaved fragments of DNA copies of viral RNA indicates that the minimum infectious dose is close to that of a double stranded copy of the viral DNA subunit (6 x 10^6 daltons). Transfection was successfully accomplished so far only in those cat, human, or baboon cells which are producing endogenous or exogenous type C virus.

A smog reactor bag was devised for measuring the mutagenicity and potential carcinogenicity of photochemical smog mixtures by circulating the gaseous material over incubating Ames tester bacteria.

Risk to human breast cancer is associated with elevated plasma estrogen and prolectin levels in adolescence. Risk of nasopharyngeal carcinoma is associated with inhaled carcinogens of an occupational or residential nontobacco, nature. EBV antibody titers are elevated in patients with squamous cell carcinoma in all subsites of the pharynx, not just the nasopharynx. Apparently healthy relatives of multipe lymphoma families were found to have altered immunological functions. A previously reported possible association between lung cancer and air pollution in Los Angeles was not confirmed in a case-control study. The excess risk was associated with past occupation.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet and other animal cancers in a natural ecology, utilizing (1) experimental animal systems, (2) basic viral and chemical carcinogenesis studies, and (3) epidemiological profiles of cancer incidence in humans and animals in the Los Angeles Area in relation to exposure to environmental carcinogens.

Proposed Course: Continuation of (1) the search for mammalian type C virus-related genes (sarcogenes and leukogenes) and the DNAs of human cancer, including transfection studies, and eventual development of vaccines for trials in cancer patients, (2) studies of the type C virus isolated from a feral house mouse, which is responsible for naturally-occurring early lymphomas and amyotrophic lateral sclerosis-like paralysis, (3) epidemiological studies of factors influencing cancer incidence and type, (4) characterizing air pollutants, previously collected, (though no new samples of these pollutants are to be collected under this contract), and (5) procurement, growth and distribution of human and animal materials to collaborating groups within the Viral Oncology Program.

Date Contract Initiated: June 26, 1968

Current Annual Level: $2,100,000

STANFORD UNIVERSITY (NO1-CP4-3228)

Title: Virologic, Biologic and Immunologic Characterization of Hodgkin's Disease and other Human Malignant Lymphomas

Contractor's Project Director: Dr. Henry Kaplan

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To collect Hodgkin's disease (HD) tissue and serological samples for study. To cultivate human lymphoma tissue in vitro and characterize the established cell lines and clones derived from them. To investigate cultures for presence of virus, and characterize any viruses detected. To characterize immunologically the cells in relation to the host autoimmune responses.

Major Findings: A previously undescribed type C RNA virus was detected in cytoplasmic fractions and isolated from the supernatant fluids of cultures of the SU-DHL-1 human histiocytic lymphoma cell line previously established in this laboratory. The virus was indistinguishable in size and morphology from typical murine type C oncogenic viruses. Viral reverse transcriptase activity was demonstrated in particles with densities of about 1.15 and 1.21g/ml from the microsomal cytoplasmic fraction and from the culture supernatant. The enzyme was partially inhibited by antibodies to the viral reverse transcriptases of RD-114 cat virus and SSV-1 simian sarcoma virus, but not by antibodies to murine leukemia virus. Typical syncytial microplaques were induced when SU-DHL-1 cells were cocultivated with rat XC cells, but no focus indiction was observed upon cocultivation with mouse UC1-B cells. Preliminary molecular hybridization studies indicated that both SU-DHL-1 and another histiocytic lymphoma line, SU-DHL-4(2) cells, contain RNA sequences partially related to the genome of SSV-1. No evidence of infectivity, inducibility, or capacity for helper rescue of defective murine sarcoma virus genomes was detected to date in cocultivation studies with several different indicator cell lines. The virion structural proteins were radiolabeled and are currently under investigation.

The Sternberg-Reed and Hodgkin giant cells from the involved spleens of patients with Hodgkin's disease were successfully grown in culture, shown to be neoplastic by virtue of aneuploidy and heterotransplantability in nude mice, and found to have surface marker, phagocytic, and enzymatic properties indicating their origin from macrophages. Two new human cell lines, ALL-1, a T-lymphocytic line derived from an acute lymphocytic leukemia, and CCS-1, derived from a clear cell sarcoma, were established. Nine previously established histiocytic lymphoma cell lines were characterized by light microscopy, transmission and scanning electron microscopy, a battery of cytochemical tests, surface markers, and other criteria. Collectively, these tests suggest that two of the nine lines are true histiocytic cells, four are "null" cells, and three appear to be of B-lymphocyte derivation. The feeder layer and nutritional requirements for the establishment and cloning of human malignant lymphoma cell lines on agar substrates were defined, and substantial cloning efficiencies were achieved with several of the cell lines.

One of the two North American Burkitt's lymphoma cell lines established in this laboratory was found to contain the Epstein Barr Virus (EBV) genome by the EBNA test; the other line was negative. Both cell lines, however, revealed the 8q-/14q+ chromosomal translocation, suggesting that the chromosomal abnormality is linked to the lymphomatous state of the cell rather than to the EBV genome. All nine histiocytic lymphoma cell lines were negative by

the EBNA test. Detailed cytogenetic studies of the histiocytic lymphoma cell lines are currently in progress.

Studies of the mechanism of impairment of cell-mediated immunity in patients with Hodgkin's disease previously revealed a reduced capacity of their T-lymphocytes to form spontaneous (E)-rosettes in vitro, as well as a decreased ability to respond to phytohemagglutinin. A rosette-inhibitory factor has now been demonstrated in the sera, spleens, and supernatants of spleen cell cultures from patients with Hodgkins's disease. Although this rosette inhibitory factor acts on normal cells, as well as on the T-lymphocytes of patients with Hodgkin's disease, the latter cells are 1,000 to 10,000-fold more sensitive than the normal lymphocytes. Binding of the rosette inhibitory factor to such lymphocytes is reversible, and rosette formation is restored to normal levels after incubation with fetal human or calf sera. The rosette inhibitory factor has been partially purified and appears to be a low-density lipoprotein complexed with C-reactive protein.

Serial studies of immune functions of patients with Hodgkin's disease, treated successfully with radiotherapy and in continuous complete remission for periods ranging from 1 to 10+ years, indicate that certain T-lymphocyte functions remain significantly impaired for several years.

Significance to Biomedical Research and the Program of the Institute: Hodgkins disease has the attributes of an infectious process. This project focuses available technology to detect evidence for a virus etiology in this malignancy. These studies may provide new information applicable to diagnosis, prognosis and treatment of Hodgkin's disease.

Proposed Course: The project will continue studies to further characterize the type C virus from the SU-DHL-1 cell line, to determine its oncogenic properties, and to investigate its relationship to HD.

Attempts will be made to convert HD cell cultures to permanent monotypic cell lines, using in vitro-in vivo selection techniques. It is felt that such monotypic cell lines could be used to induce heterologous antibodies, possibly of use for determining tumor-specific antigens shared with other HD cells. Attempts will be made to infect EBNA-negative HD cells with EBV by cocultivation. Once immediate cell cultivation goals have been achieved, attention will be turned to establishing cell cultures of other types of human malignant lymphomas which have not as yet been successfully cultivated in vitro.

Attempts will be made to purify the low-density lipoprotein-C-reactive protein complex which exhibits rosette-inhibitory reactivity to determine in which moiety of the protein complex the activity resides. Investigation will be carried out on this factor for effects on other types of immunologic responses in vitro. It is hoped that these studies will have relevance to the impairment of cell-mediated immunity observed in HD patients.

Date Contract Initiated: April 1, 1974

Current Annual Level: $375,000

TENNESSEE, UNIVERSITY OF (NO1-CP4-3325)

Title: Immunological Studies on the Relationship of Embryonic Antigens to Virus-Induced Tumor Antigens

Contractor's Project Director: Dr. Joseph H. Coggin

Project Officers (NCI): Dr. Phyllis Brandchaft

Objectives: To apply immunological approaches to determine the relationship between virus-coded, tumor-specific transplantation antigens (TSTA) on the surface of cells transformed by oncornaviruses, particularly SV40 and adenoviruses, and other transplantation antigens such as embryonic antigens coded by the cellular genome.

Major Findings: Two new mutants of SV40-induced tumor cells were obtained which are unique in that the EA^- state seems stable and current work shows that the $TSTA^+$ state is retained in these mutants. These cells will be used for comparative studies with EA^+, $TSTA^+$ SV40 induced tumor cells.

Cell-free preparations of SV40 cell-surface antigen were isolated which are capable of eliciting solid immunity to SV40 tumor challenge and which effectively interrupt SV40 oncogenesis in vivo. The preparations can be prepared by one of several methods including saline extraction and the use of 3m KCl. Semi-purified preparations of the cell extract when given in multiple injections to adult hamsters confer strong enhancement when the animals are subsequently challenged with SV40 induced sarcoma cells of a syngeneic line. If, however, the animals receive a single dose of antigen carefully standardized for protein concentration, the animals show solid immunity to challenge. These data confirm the work of Pellis, Kahan et al. that antigens removed form chemically-induced tumors, and now for SV40 tumors, are capable of eliciting strong transplantation immunity when given in cell-free soluble form, if the amount of antigen presented to the animals in a single exposure is carefully controlled. Animals immunized with single doses of the cell membrane extract are able to yield lymphocytes which can confer strong immunity to normal animals following transfer of syngeneic immune lymphocytes and subsequently challenged with living SV40 tumor cells. A similar antigen preparation has been isolated from fetal cells of mid-gestation of the hamster in an effort to now purify the antigen from fetal membranes. Large batches of the semi-purified antigen have been prepared and are being chemically and biologically analyzed in collaborative work.

The spontaneous hamster lymphoma disease which occurred in high incidence in the hamster colony could be passaged with filtered extracts of lymphoma tissue. Animals receiving these filtrates generally develop a high percentage of tumors. The disease was established in hamsters housed in a mobile facility so that the causative agent could be pursued more aggressively. The disease can be prevented in hamsters by sensitizing the animals to extracts of spontaneous lymphoma cells or by immunization of the animals with irradiated transplantable lymphoma cells prior to exposure of the animals to the infected area. The immunity is directed against cell membrane antigen and can be passively transferred from an immunized animal to a normal animal by peritoneal exudate.

Studies are underway to detail the route of infection for the naturally occurring disease and for isolating the agent responsible.

Significance to Biomedical Research and the Program of the Institute: The hypothesis that derepressed host genes are respnsible for oncogenesis is being actively studied by several groups in cancer-related programs. The relationship between fetal antigens and viral-induced antigens is an important issue in relation to the question of etiology as well as to programs concerned with prevention and control of virus-associated cancers. To better understand the mechanism of neoplastic transformation, it is essential to distinguish between host gene re-expression and new viral gene products.

Proposed Course: This contract terminated on June 27, 1977.

Date Contract Initiated: June 28, 1974

Current Annual Level: $146,807

FUNDACION PARA LA EDUCACION SUPERIOR (NO1-CP6-1037)

Title: HL-A and Lymphoma Case Control Study in Colombia

Contractor's Project Director: Dr. Alvaro Duenas

Project Officer (NCI): Dr. Ernest Plata

Objectives: To explore the role of Epstein-Barr Virus and other viral agents which may play a role in the etiology of childhood Hodgkin's-like lymphomas found in Colombia, South America. To study genetic markers (HL-A, ABO blood groups) and nutritional factors which may have a bearing on the susceptibility to this disease. To study the immune competence of patients.

Major Findings: No progress report has been received for this project.

Significance to Biomedical Research and the Program of the Institute: This project was initiated to study the particularly aggressive form of early Hodgkin's lymphoma which is found in Colombia. It was hoped viruses, genetic susceptibility markers, or nutritional factors involved in the etiology of the disease could be identified.

Proposed Course: This contract was terminated June 29, 1977.

Date Contract Initiated: June 30, 1976

Current Annual Level: $19,500

SUMMARY REPORT

(3) COCARCINOGENESIS STUDIES SECTION

July 1, 1976 through September 30, 1977

There is overwhelming evidence that many environmental carcinogens (biological, chemical, or physical) produce an increase in tumor risk. In view of the strength of the association between exposure to carcinogens and the development of neoplasia in animals and man, increased emphasis is being placed on determining modes of action of numerous carcinogens. Although the initiating event may be a "single hit" phenomenon, the bulk of the evidence suggests a multifactorial etiology in the promotion of the neoplastic process. In this regard, there is increasing evidence that the actions of several classes of carcinogens are mediated by the activation of latent viruses or endogenous viral genes in cells of laboratory animals. In order to further an understanding of the mechanisms of carcinogenesis, the Viral Oncology Program has recently established the Cocarcinogenesis Studies Section (CSS) which will sponsor extramural studies on the nature and the effects of interactions of biological, chemical, or physical environmental factors with cancer viruses and/or intracellular viral genetic information. The areas of investigation to be pursued in cocarcinogenesis are briefly described as follows:

Systems Development: The development and standardization of cell-virus transformation assay systems for use as research tools, or as reliable, sensitive systems for *in vitro* carcinogen testing.

Establish Cofactorial Interactions: Provide further evidence of the cofactorial action of multiple viruses and of viruses and other environmental carcinogens in inducing neoplastic transformation of mammalian cells; and correlate the mutagenic and carcinogenic effects of biological, chemical and physical environmental factors with enhanced expression of virus genetic information contained in such cells.

Mechanistic Studies: Investigate the nature and determine the products of the interactions between endogenous viruses and/or intracellular viral genetic information and environmental stressors which result in neoplastic transformation.

Control Technology: As a correlary of mechanistic studies, define methods which may prevent or control the development of neoplasia resulting from the interaction of viruses with chemical, physical or other biological environmental factors. Eight of the ten contracts presently assigned to the CSS were initiated in late FY 76 or early FY 77. As a consequence, only preliminary results are now available.

A. Systems Development

The development of reliable, sensitive, standardized in vitro cell-virus transformation assay systems for use as research tools is perceived as one of high priority.

Presently under development and testing or in use for mechanistic studies are cell cultures derived from (1) murine species (normal Balb/c, C57L, C3H, NIH Swiss AKR mice; ^{90}Sr-induced osteosarcoma of CF#1; fetal rat liver; hamster embryo; Sprague-Dawley and Fischer rat; (2) subhuman primates (baboon, gibbon ape and rhesus monkey); and (3) human origin (human osteoma-HOS; normal embryonic skin, skin-muscle, lung, thyroid, brain, cervix-uterus, prostate, splenic "B" lymphocytes and skin of individuals expressing Lesch-Nyhan/Blooms Syndrome and xeroderma pigmentosum. Emphasis is being placed on the development and use of primate-derived cell cultures in an attempt to establish assay systems possibly more relevant to the human situation. Although some cell strains or lines derived from some of the above sources have been is use for considerable periods of time, it cannot yet be ascertained whether others may prove valuable as research tools.

B. Establish Cofactorial Interactions and Study Mechanisms of Transformation

The majority of the cocarcinogenesis contracts have been underway for only five months. However, preliminary findings suggest that several cell-virus systems may prove useful for establishing the role of environmental carcinogens and viruses as they interact to induce neoplastic transformation of mammalian cells. Preliminary findings which lend encouragement to the possibilities of studying mechanisms of action include the observations that (1) transformation of Sprague-Dawley fibroblast cells is accelerated by a single treatment with chemical carcinogens; (2) virus has been isolated from tumor cell cultures derived from ^{90}Sr-induced osteosarcomas of CF#1 mice; (3) morphologic transformation of hamster and rat embryo fibroblasts by adenovirus 12 is enhanced by pretreatment of cell cultures with N-methyl-N-nitrosourea (NMU); (4) morphologic transformation of HOS cells (derived from a human osteoma) has been achieved using herpes simplex virus type 2; (5) treatment of HOS cells with NMU has yielded clones of morphologically transformed cells; (6) morphologic transformation of NIH Swiss mouse whole embryo cultures has been accelerated by treatment with methylcholanthrene; (7) specific "sarcogene" probes have been produced to search for products of virus transcription and translation in repositories of normal and transformed cells; and (8) procedures have been developed which permit isolation and assay for the presence of baboon endogenous virus.

Experimental results indicate that the Fischer rat embryo fibroblast cell line F1706 is useful as a screen for potential carcinogens and for follow-on mechanistic studies. Morphological transformants possessing the ability to grow in soft agar have been produced using N-methyl-N-nitrosourea (NMU), Aflatoxin B_1, fourteen coded smog fractions, trichlorethylene (TCE), methylene chloride, tetrachloroethylene and trichloroethane. Transformants produced by the last four named chemicals have been inoculated into newborn

rats to confirm tumorigenicity. The results of transformation of F1706 cells are also being correlated with mutagenesis of S. typhimurium.

Two murine cell lines, C_3H 10T½ C18 and Balb 3T3 C1 A31, are being used in an attempt to develop systems useful for discriminating potential carcinogens and for studying the role of type C RNA virus expression in chemical carcinogenesis. Virus expression can be induced in these cell lines by treating cultures with IUdR and dexamethasone.

It has been shown that human epithelial cells have five-fold or more AHH activity than fibroblasts from the same donor. MCA treatment of mixed epithelial and fibroblast cell cultures, but not pure fibroblasts, led to actively growing fibroblastic cultures with a large heteroploid population.

Preliminary data on spontaneous x-tropic virus expression in crosses between NZB X 129 mouse strains and between NZB X SWR mouse strains suggests that a single autosomal dominant gene is responsible for the regulation of x-tropic virus expression. The sensitivity to 3-methyl-cholanthrene (MCA) induced fibrosarcomas in these strains is correlated with the level of x-tropic virus expression. These in vivo systems will be further utilized to determine the role of x-tropic virus in 3-MCA-induced tumors of mice.

C. Control Technology

It is apparent that a number of intrinsic (host) factors and extrinsic (environmental) factors interact to control virus expression and malignant transformation of mammalian cells in vitro and in vivo. Assuming that viruses are cofactorially involved in chemical carcinogenesis, it has been postulated that the suppression of virus production or viral gene expression may result in suppression of carcinogen-induced neoplasia. In the past few years, several lines of evidence have been developed which lend credence to the possibility of controlling spontaneous or carcinogen-induced neoplasia through the use of active or passive immunization with virus-specific products. Such control studies are a natural correlary of mechanistic studies and are dependent upon the use of suitable in vivo systems and the development and use of in vitro cell-virus transformation assay systems. A primary objective of each of the recently initiated projects is to develop such in vitro systems.

Certain cell systems, primarily Fischer rat embryo cell lines, presently are being used to study potential anti-cancer products including viral vaccines and virus-specific antibody, interferon and viral inhibitors. It has recently been shown that Geldanamycin (Gld) not only protects F1706 cells from transformation by 3-MCA but that cells protected from transformation by Gld were atumorigenic in newborn rats.

Anti-RaLV IgG added two or three days prior to treatment of cells with 4-nitroquinoline oxide (4-NQO) protects Fischer rat embryo cells from phenotypic transformation and oncogenicity for the newborn rat. The additions of IgG at the same time as or after treatment with 4-NQO exerted no such protective effect. Studies to determine whether the cellular toxicity of the IgG is related to its ability to inhibit transformation are now in progress.

Preliminary results indicate that retinoic acid (vitamin A) may reduce the ratio of transformed colonies resulting from the treatment of F1706 cells with 3-MCA.

COCARCINOGENESIS STUDIES SECTION

Dr. James L. McQueen, Head

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1 CP3-3249)

Title: Genetic and Immunologic Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. John Stephenson

Objectives: To elucidate the processes of host cell restriction of viral replication, determine host immune respnses to viral products of infection and evaluate their influence on the susceptibility of different strains of mice to spontaneous or induced viral leukemogenesis.

Major Findings: (1) Mechanism of the H-2 Effect on Viral Leukemogenesis. As in several other systems, Friend virus (FV)-infected cells can induce T killer cells in syngeneic hosts which show the phenomemon of H-2 restriction. Experimental evidence strongly suggests that this phenomenon is based on the existence on cell surfaces of molecular complexes formed selectively between certain H-2K or H-2D molecules and certain viral molecules (probably gp69). Evidence for this conclusion is from studies of (i) blocking of T killer cell activity with specific anti-H-2 sera, (ii) cocapping experiments, and (iii) the finding of selective rather than random inclusion of specific H-2 molecules into virus particles. Further evidence, still inconclusive, suggests that H-2 type influences the virus-producer or non-producer status of FV tumor cells. (2) Mechanism of Fv-2 Resistance to FV. Earlier results suggesting that Fv-2^r mice are blocked at the level of malignant erythropoiesis in their response to FV have been extended to studies comparing semi-congenic Fv-2^s and Fv-2^r mice with respect to ^{59}Fe uptake and formation of erythropoietin-independent erythroid colonies (CFU-E). The results of these studies do not bear out the hypothesis of blocked malignant erythropoiesis; rather they suggest that the primary defect in Fv-2^r mice might be at the level of capacity to permit virus replication. Another new finding is that Fv-2^r appears to be a pre-weaning lethal gene in the homozygous condition on the DBA/2 background but not on the C57BL background. (3) Genetic Suppression of AKR Spontaneous Leukemia. RF, DBA/2 and C3HeB mice each possess a dominant gene(s) which suppresses leukemia in crosses with AKR and C58 mice. In the RF strain, it appears to be a single gene, perhaps a variant of Fv-1^n. Although the gene(s) suppresses leukemia, it does not suppress the expression of ecotropic MuLV at 6 weeks of age. (4) Genetic Factors in Chemically Induced Leukemia. Strain distribution studies and some preliminary genetic analyses indicate that the marked polymorphism in the response of mice to methylcholanthrene (MCA) painting (skin tumors vs. leukemia) is due to the Ah gene which controls the enzyme, aryl hydrocarbon hydroxylase. In addition, evidence for the involvement of endocrine factors comes from the observation that females of Ah^d strains are much more susceptible to MCA-induced leukemia than males, but the resistance of the males is abolished by prior treatment with cortisone.

Significance to Biomedical Research and the Program of the Institute: By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanisms governed by individual genetic loci, with the objective, eventually, of increasing or altering immunogenetic effects to provide maximum resistance against cancer. The identification of markers associated with cancer resistance and susceptibility could prove important in identifying cancer-susceptible individuals or in early diagnosis of the idsease.

Proposed Course: Emphasis will be placed on the study of viral and genetic factors in influencing chemically-induced leukemia, especially in RF mice.

Date Contract Initiated: May 13, 1965

Current Annual Level: $124,990

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (YO1-CP7-0503)

Title: In Vitro Interaction of Chemical Carcinogens with Primate Cells: Relationship to Expression of Endogenous Primate or Exogenous Murine Viruses

Contractor's Project Director: Dr. George C. Lavelle

Project Officer (NCI): Dr. Stephen Tronick

Objective: To obtain new information on the importance of the expression of oncornavirus genes in chemically-induced transformation events in primate cells, the contractor will: (1) establish an in vitro transformation assay system utilizing diploid fetal baboon cells; (2) determine if chemical carcinogens have a potentiating effect on transformation by oncornaviruses in primate cells and correlate such transformation with the expression of baboon endogenous virus and (3) determine the effect of chemical carcinogens on primate cells expressing xenotropic oncornavirus.

Major Findings: The approach involves measuring for expression of baboon endogenous virus (BEV) or xenotropic viruses in baboon cells exposed to chemical carcinogens and/or inhibitors of protein synthesis. One secondary line of whole baboon embryo has been found to spontaneously release BEV. Host range properties of that virus have been established in some human and mink cell lines and procedures have been developed for virus assay.

Significance to Biomedical Research and the Program of the Institute: This project will be directed at the development of primate-derived cell-virus transformation assay systems in order to provide basic data on the potentiation by endogenous and xenotropic viruses of chemical carcinogenesis in primates.

Proposed Course: Continuation of activities.

Date Contract Initiated: November 1, 1977

Current Annual Level: $124,800

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CP7-0504)

Title: In Vivo Radiation-Activation of Endogenous Sarcoma Virus Genome

Contractor's Project Director: Dr. Emerson Chan

Project Officer (NCI): Dr. John Dahlberg

Objectives: To ascertain the cofactorial role of oncornaviruses in the induction of malignancies in mice by ionizing radiation, the contractor will: (1) characterize the FBJ and FBR murine viruses and determine the identity and nature of sarcogene sequences in normal mice tissues and radiation-induced osteosarcomas and (2) determine whether activation of endogenous sarcogenes/virogenes by radiation is an underlying mechanism of the neoplastic process.

Major Findings: The contractor is characterizing the FBJ and FBR viruses (isolated from radiation-induced and spontaneously occurring neoplasms in mice) and using ^{3}H-cDNA probes to establish whether the viruses are true endogenous murine viruses. Attempts are being made to isolate and characterize viruses from ^{90}Sr-induced bone tumors in mice. The host range of FBJ and FBR viruses appears limited to murine cells. Balb/c cells are being used for large-scale virus production. Preliminary data, using ^{3}H-cDNA probes of RLV and FBJ viruses indicates substantial difference in viral nucleotide sequence homology. Additional virus isolates have been obtained from in vitro cultures of ^{90}Sr-induced osteosarcoma in CF#1 mice.

Significance to Biomedical Research and the Program of the Institute: This project will further identify and characterize endogenous murine viruses and elucidate the role of oncornaviruses in the radiation-induced oncogenic process. These studies will extend the knowledge by which radiation induces neoplasia in animals and man by determining the specific role of putative endogenous type C oncornaviruses in radionuclide-induced bone cancer in mice.

Proposed Course: Continuation of activities.

Date Contract Initiated: November 1, 1976

Current Annual Level: $200,647

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP4-3240)

Title: Viral-Chemical Carcinogenesis Studies

Contractor's Project Director: Dr. Paul Price

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To develop, standardize and apply in vitro systems for studying viral-chemical cocarcinogenesis using known and suspected carcinogens in the environment. Utilize and complement in vitro systems to elucidate viral and cellular components in cell transformation, including the role of endogenous viruses. Utilize cell systems to study potential anti-cancer products, including viral vaccines, viral inhibitors, etc., to elucidate the role of type C viruses in transformation. Utilize and adapt subhuman transformation and viral induction procedures to studies of human tumor and transformed cell lines for assaying suspected chemical carcinogens. Study the effect of viral vaccines, viral inhibitors, interferon and/or genetic regulation of endogenous virus on naturally occurring and chemically induced tumors.

Major Findings: It has been shown that N-Methyl-N-nitrosourea (NMU), aflatoxin B_1 and 14 of 20 smog fractions cause transformation of F1706 cells. The transformed cells grew in soft agar. Another smog sample transformed the cells but the transformed cells did not grow in soft agar. Twelve clones of H8324 (Fischer rat embryo) have been isolated for possible future use in transformation studies. It has been shown that Trichlorethylene (TCE) is a transforming agent but is possibly less oncogenic than three alternatives being considered by industry. The transformed cells have been inoculated into newborn Fischer rats for confirmation of the results. It has been shown that twice washed purified microsomes produce biologically active metabolites from benzo(a)pyrene (BP) within a 120 minute incubation time. The usefulness of this activation system in detecting BP-induced transformation is currently under test in several laboratories at Microbiological Associates, Inc., using 10T 1/2, BALB 3T3 and F1708 cells.

It was previously reported that Geldanamycin (Gld) protects F1706 from 3-methylcholanthrene (3-MCA)-induced transformation. This has now been confirmed by showing that the protected cells were non-tumorigenic in newborn Fischer rats. It has been shown that anti-rat leukemia virus (FaLV) IgG added 3 or 2 days before treatment with 4-nitroquinoline (4-NQO) protects the cells from transformation. When the IgG was added 16 hours before 4-NQO the time to transformation was increased but transformation was not prevented. There was no protection when IgG and 4-NQO were added at the same time. When IgG was added after 4-NQO, it appeared to act as a promotor of transformation. Various absorption studies were conducted in an effort to discriminate between the toxic and virus neutralizing properties of IgG. The optimum was found to by 3 absorptions of RaLV IgG against rat rbc's.

Preliminary results obtained with M-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and benzo(a)pyrene (BP) indicate that the H12 cell line may be superior to F1706 for developing a mammalian cell mutagenesis assay. Cumulative data continue to show a high degree of correlation between bacterial mutagenesis, in vitro transformation of F1706 cells and in vivo carcinogenesis. Completed data are now available on 25 compounds and another 21 are in various stages of testing.

It has been shown that human epithelial cells have 5-fold or more arylhydrocarbon hydroxylase (AHH) activity than fibroblasts from the same donor. This activity is lost rapidly upon physical subdivision. MCA treatment of mixed cultures, but not pure fibroblasts, led to actively growing fibroblastic cultures with a large heteroploid population.

Unique chromosome markers were identified in several of the heteroploid cell strains.

Cumulative data suggest that a single autosomal dominant gene is responsible for the regulation of X-tropic virus expression and that there is no significant difference in "neutralizing factor" (NF) level among the various crosses and backcrosses. Crosses with high X-tropic virus expression are more sensitive to MCA-induced fibrosarcomas than are animals with low virus expression. The titer of NF in C57BL/6 mice at the time of MCA treatment and one month after treatment were the same.

Cell cultures of interest to this contract are characterized, frozen and stored by the contractor. Frozen cells are reconstituted and shipped to others in the Virus Cancer Program upon request.

Significance to Biomedical Research and the Program of the Institute: This contract has established the feasibility of using tissue culture systems for screening environmental compounds for carcinogenic potential in a fraction of the time and cost of screening in animals. Thus, within weeks, rather than months or years, one could test for carcinogenic potential, and only those compounds which transform cells would need to be confirmed in animals. The tissue culture systems developed also permit screening of many times the number of compounds possible in animals. Since over 20,000 compounds reach the market each year, with a backlog of more than 100,000, the need for such systems is very urgent. Progress has been made in expanding in vitro systems useful for mutagenesis-carcinogenesis assays.

The development of materials and methods for active or passive immunization against carcinogenesis have important implications for cancer prevention and control. The results using mouse interferon against chemically induced tumors in animals and the inhibition by IgG of chemically induced transformation in vitro lend encouragement to such a method of prevention or control.

Proposed Course: Work will be continued on (1) the development, evaluation and standardization of in vivo and in vitro systems for studying the effects of known and suspected environmental carcinogens; (2) development and application of sensitive in vitro assay systems for studying the natural history of type C tumor virus infection and its rlationship to carcinogenesis in a variety of mammalian species (3) development and testing of type C virus vaccines, interferon and anti-transformation compounds to evaluate their efficacy in prevention of spontaneous and chemically-induced tumors in genetically defined mouse strains and cell systems.

Date Contract Initiated: February 1, 1970

Current Annual Level: $798,763

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP6-1043)

Title: In Vitro Malignant Transformation of Human and Subhuman Primate Cells by Interaction between Viruses and Chemicals

Contractor's Project Director: Dr. Aaron Freeman

Project Officer (NCI): Dr. Robert Bassin

Objectives: To determine whether chemical carcinogens enhance or potentiate viral oncogenic transformation of primate cells in vitro and to elucidate the mechanisms of action, the contractor will: (1) treat human or nonhuman primate cell cultures persistently infected with oncogenic virus with chemical carcinogens and observe for and confirm malignant transformation events; (2) develop appropriate probes for detection of sarcogene expression; and (3) investigate the mechanisms of interaction to determine the target reaction(s) involved in sarcogene expression and malignant transformation.

Major Findings: Attempts are being made to chronically infect cell cultures of human epithelial and fibroblast cells and similar cell cultures from nonhuman primates (marmosets) with simian sarcoma virus SSV (SSAV), gibbon ape leukemia virus (GALV) and baboon endogenous virus (BEV) and to establish reliable transformation assay systems from such cell/virus combinations. Cultures treated with 3-methylcholanthrene and N-methyl-N'-nitro-N-nitrosoguanidine are monitored for transformation. Using appropriate cDNA and immunologic probes, the contractor will test whether sarcogene information is expressed in the transformed cells. Preliminary results indicate that a human foreskin fibroblast cell strain has been infected with baboon endogenous virus. Toxicities of 4-nitroquinoline and 3-methycholanthrene for human fibroblasts have been determined. Transformation induction experiments have been initiated.

Significance to Biomedical Research and the Program of the Institute: The cocarcinogenic effects of C type RNA viruses and chemical carcinogens has been demonstrated in rodent cell systems. This leads to the hypothesis that "activation" of cellular sarcogenes or oncogenes is involved in the development of malignant transformation. Although neither human type C viruses nor endogenous sarcogenes have been isolated from primate cells, many analogies strongly suggest that such entities exist. This effort will probe for the presence of such entities and could result in the rescue of primate sarcogenes. These in vitro studies may result in the establishment of relevant, rapid and inexpensive test systems for identifying environmental carcinogens with potential for causing human cancer.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 29, 1976

Current Annual Level: $205,157

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP6-1045)

Title: Development of Mammalian Cell Lines Known to Contain Endogenous Oncogenic Virus Sequences which Can be Utilized in Testing Mutagenic and Carcinogenic Effects of Environmental Factors

Contractor's Project Director: Dr. Paul Price

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To develop cell lines (or strains) from species known to contain integrated oncornavirus sequences and to use such in vitro systems in cocarcinogenesis assay, the contractor will: (1) develop in vitro cell lines known to contain integrated oncornavirus sequences; (2) determine transformation frequencies when exposed (and not exposed) to chemical or physical carcinogens; (3) confirm malignant transformation of morphologically altered cells; (4) monitor endogenous sarcogene expression with suitable probes; and (5) correlate mutagenic and carcinogenic activities with each other and determine the mechanisms of action and target sites for the carcinogens tested.

Major Findings: The contractor is extending the expertise gained with the Fischer rat embryo F111 cell system by attempting to develop primate transformation assay systems from human skin cultures and a human differentiated splenic cell line (B-lymphocyte) free of or chronically infected with the X-tropic baboon endogenous virus and/or the ecotropic feline leukemia virus. Cell strains or lines presently being used include normal human skin, the human splenic B-lymphocyte and three strains from individuals expressing genetic anomalies. Toxicities of Benzo(a) pyrene and N-methyl-N'-nitrosoquanidine have been determined. Attempts have been made to infect the cell lines with feline, gibbon ape and baboon type C viruses. Attempts are being made to establish a system for selection of ouabain resistant cell lines.

Significance to Biomedical Research and the Program of the Institute: The Fischer rat embryo F111 cell culture transformation assay system has been used for identifying chemical carcinogens in double blind studies. A role played by the virus in viral/chemical induced transformation has also been suggested by demonstrating that virus-specific antibody protects the cells against transformation by potent carcinogens. Successful extension of these results to primate cell lines might yield an assay system more relevant to the human situation as well as providing important and vital information on the basic biology of malignant transformation.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 29, 1976

Current Annual Level: $231,779

PENNSYLVANIA STATE UNIVERSITY (NO1-CP6-1063)

Title: In Vitro Malignant Transformation of Human and Subhuman Primate Cells by Interaction Between Viruses and Chemicals

Contractor's Project Director: Dr. Fred Rapp

Project Officer (NCI): Dr. Masakazu Hatanaka

Objectives: To determine whether chemical carcinogens enhance or potentiate viral oncogenic transformation of primate cells in vitro and to elucidate the mechanisms of action, the contractor will: (1) treat human or nonhuman primate cell cultures persistently infected with virus with chemical carcinogens and observe for and confirm malignant transformation events; (2) develop appropriate probes for expression of sarcogene expression; and (3) investigate the mechanisms of interaction to determine the target reaction(s) involved in sarcogene expression and malignant transformation.

Major Findings: Various cell strains or lines exposed to DNA viruses (HSV-1, HSV-2, CMV, EBV, SV40, Ad) are being pretreated, cotreated or treated after infection with N-nitroso-compounds (primarily NMU and NEU) and observed for transformation events. Transformed cells will be tested for oncogenicity in nude mice. Transformed clones also will be examined to determine whether they are expressing type C virus information. Any emerging type C virus populations will be examined to determine the host specificity.

Investigation of synergism between N-nitroso compounds and oncogenic viruses in the potentiation of in vitro transformation has been initiated.

Utilization of a quantitative assay for herpesvirus transformation has demonstrated that high doses of NMU inhibit cell division and as a result inhibit the "fixation" of transformation. Lower doses of NMU have enhanced virus transformation and experiments are presently in progress to optimize the dose. Enhancement of virus transformation has been achieved when the cells are permitted to "recover" from drug treatment in normal medium prior to virus infection.

To examine cocarcinogenesis in human or primate transformation systems, assays for the transformation of the human osteoma cell line, HOS, have been established. Subclones of HOS cells are being selected for ability to grow in bromodeoxyuridine. Since herpes simplex virus (HSV) carries a virus specific thymidine kinase (TK), thymidine kinaseless (TK^-) cells can be "transformed" by the virus to the TK^+ phenotype. It has been demonstrated that HOS cells are not tumorigenic in nude mice and do not grow in soft agar. These parameters will be useful in measuring transformation. A morphologic transformation system of human cells by HSV has also been developed and is being quantitated for use in carcinogen enhancement studies.

Separate attempts to measure drug-mediated transformation of the HOS line have yielded clones of cells which appear morphologically transformed after exposure to high doses of NMU. If these cells prove to be oncogenic (by inoculation into nude mice) this will represent the first in vitro transformation of a human cell by N-nitroso compounds.

Enhancement of adenovirus transformation by NMU is being examined with the well-characterized rat and hamster embryo system and will be extended to primary monkey cells and the HOS line. Transformation of rat embryo cells by adenovirus 12 is enhanced by exposure to 0.1mM NMU up to 12 hrs prior to infection.

Significance to Biomedical Research and the Program of the Institute: Several DNA viruses, notably the papovaviruses, herpesviruses and adenoviruses have been shown to possess transforming or oncogenic potential in laboratory animals. This contractor will attempt to determine whether a synergistic effect exists between carcinogens and DNA viruses in the process of malignant transformation. The effort also may provide insights into whether DNA tumor viruses mediate cellular transformation by activating endogenous host oncogenic sequences. These studies may provide in vitro test systems vitally needed for rapid screening of potential carcinogens.

Proposed Course: Continuation of activities of new contract.

Date Contract Initiated: September 29, 1976

Current Annual Level: $65,973

ST LOUIS UNIVERSITY (NO1-CP6-1062)

Title: Influence of Interactions Between Environmental Factors (and Viral Sarcogenes in Malignant Transformation of Murine Fibroblasts)

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Stephen Tronick

Objectives: To further elicidate the cofactorial relationships between carcinogens (chemical, physical, and/or biological) and endogenous type C RNA viruses as these relate to activation of endogenous "sarcogene" expression and malignant transformation of cells in vitro, the contractor will: (1) determine the correlation between sarcogene activation by carcinogens and malignant transformation of cells, and (2) determine the nature and mechanisms of the interaction of the carcinogenic cofactor with the cell which leads to activation of the cellular/viral genetic information.

Major Findings: The contractor is investigating the relationship between endogenous sarcogene expression and/or the expression of type C viruses in murine cells derived from low (C57L or NIH Swiss), moderate (BALB/c), and high (AKR or C58) leukemia incidence strains following treatment with chemical carcinogens. Sarcogene probes (^{3}H-cDNA) prepared from M-MSV (MLV) are being used to monitor and quantitatively and qualitatively measure sarcogene transcription in transformed murine cells. Morphological transformation has occurred in early passages of 3-methycholanthrene-treated NIH Swiss whole embryo cultures and in later passages of controls. A ^{3}H-cDNA probe representing only the sarcoma virus sequences of Moloney-MSV has been prepared and thoroughly characterized. This probe is being used to assay for mRNA and nuclear RNA in a variety of spontaneously transformed and virus and/or chemically induced transformed cell cultures.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemical or physical carcinogens and viruses in inducing cell transformation in vitro and tumor production in vivo have been

demonstrated. The mechanism(s) of action and the relative importance of the interaction between these putative cocarcinogens is not well understood. Because it is not feasible to eliminate most carcinogens from the environment, only a thorough understanding of the basic biology of malignant transformation, including the mechanisms by which viruses or viral genetic information and other carcinogens interact, is likely to indicate practical approaches to prevention and control of many forms of cancer.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 29, 1976

Current Annual Level: $185,332

UNIVERSITY OF SOUTHERN CALIFORNIA (NO1-CP6-1041)

Title: Influence of Interactions Between Environmental Factors (and Viral Sarcogenes in Malignant Transformation of Rat Cells)

Contractor's Project Director: Dr. Suraiya Rasheed

Project Officer (NCI): Dr. Edward M. Scolnick

Objectives: To further elucidate the cofactorial relationships between carcinogens (chemical, physical, and/or biological) and endogenous type C RNA viruses as these relate to activation of endogenous "sarcogene" expression and malignant transformation of cells in vitro the contractor will: (1) determine the correlation between sarcogene activation by carcinogens and malignant transformation of cells, and (2) determine the nature and mechanisms of the interaction of the carcinogenic cofactor with the cell which leads to activation of the cellular/viral genetic information.

Major Findings: The contractor is investigating the relationship between endogenous sarcogene and virogene expression in spontaneous and chemically (3-methylcholanthrene and N-methyl-N'-nitro-N-nitrosoquanidine) transformed Sprague-Dawley rat embryo cell cultures. Single cell clones of Sprague-Dawley fibroblast cells ranging from nonvirus producing to virus producing normal and transformed cells have been obtained. Transformation is accelerated by carcinogen treatment. Cross molecular hybridization studies using ^{3}H-cDNA probes from Sprague-Dawley rat leukemia virus (SD-RaLV) and Kirsten murine sarcoma viruses are in progress. All results are preliminary.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemical or physical carcinogens and viruses in inducing cell transformation in vitro and tumor production in vivo have been demonstrated. The mechanism(s) of action and the relative importance of the interaction between these putative cocarcinogens is not well understood. Because it is not feasible to eliminate most carcinogens from the environment, only a thorough understanding of the basic biology of malignant transformation,

including the mechanisms by which viruses or viral genetic information and other carcinogens interact is likely to indicate credible approaches to prevention and control of many forms of cancer.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 29, 1976

Current Annual Level: $90,055

UNIVERSITY OF TEXAS MEDICAL BRANCH (NO1-CP6-1042)

Title: In Vitro Transformation of Mammalian Cells Resulting from the Intra-Cellular Interaction of a Non-oncogenic Virus and Chemicals

Contractor's Project Director: Dr. Thomas Albrecht

Project Officer (NCI): Dr. Robert Goldberg

Objectives: To explore the possibility that interactions between chemical carcinogens and cells persistently infected with a non-oncogenic virus may result in malignant transformation the contractor will: (1) develop in vitro cell culture systems persistently infected with normally non-oncogenic viruses; (2) subject such cultures to insult by carcinogenic chemicals; (3) monitor treated and control cell cultures for morphological transformation, activated expression of endogenous oncornavirus information and altered expression of the non-oncogenic virus; and (4) confirm the malignant potential of transformed cells by in vivo innoculation of appropriate host.

Major Findings: The contractor is using several strains of cytomegalovirus (CMV) and several varicella-zoster viruses in cell cultures derived from human fetal skin-muscle, lung, thyroid, brain, cervix, prostate, bladder and bowel in an attempt to establish persistently infected cell lines utilizable as quantitative transformation assay systems. Studies on plaque characteristics of five laboratory strains of CMV in human embryonic skin-muscle, lung and thyroid cells have been completed. New isolates of CMV and varicella/zoster have been obtained. Toxicity tests are being conducted of thymus cells persistently infected with the five laboratory strains of CMV. Transformation experiments of thymus and lung cells have been initiated.

Significance to Biomedical Research and the Program of the Institute: The synergistic effects of chemicals and oncogenic viruses in malignant transformation of cells has been demonstrated. Similarly, several normally non-oncogenic DNA viruses have been shown to be oncogenic after treatment by chemical or physical factors. This project will further the understanding of the possible importance of the interaction of chemical carcinogens and normally non-oncogenic viruses as related to malignant transformation.

Proposed Course: Continuation of activities.

Date Contract Initiated: September 27, 1976

Current Annual Level: $103,451

SUMMARY REPORT

(4) DNA VIRUS STUDIES SECTION

July 1, 1976 through September 30, 1977

The principal objective of the DNA Virus Studies Section is the determination of the etiological relationships of DNA viruses to human malignancy. Studies leading to this objective are carried out through research contracts managed by the DNA Virus Studies Section. The program of the Section on potentially oncogenic DNA viruses is divided into two major categories: (1) studies of DNA virus relationships to human disease; and (2) studies in animal model systems. The first category is subdivided into three parts, each identified with specific viruses. Thus, this category deals with, first, the Epstein-Barr virus (EBV), second, herpes simplex virus (HSV), and, third, small DNA viruses, as papovaviruses and adenoviruses. The second category includes studies of herpesviruses in nonhuman primates and in lower vertebrates.

EBV Studies: Following purification and concentration of the EBV nuclear antigen (EBNA), the antibody binding ability of EBNA was shown to be dependent on the presence of nonspecific DNA, EBNA sub-type differences were defined by elution characteristics and immunological evidence, and the first direct proof was obtained that the complement-fixing(S) antigen and EBNA are identical.

Cell lines, previously considered as nonproducers, were shown to produce viral antigen in a small number of cells. Induction of early antigen (EA) with ultraviolet-inactivated virus in EBV-genome negative cell lines confirmed the postulate that the resident EBV-genome participates in the induction.

Nasopharyngeal carcinoma (NPC) cell/mouse fibroblast hybrids provided both evidence implicating chromosome 21 and the first definite NPC cell derivative, in vitro, useful for direct study.

BCG selectively stimulated the anti-membrane EBV-specified antigen (MA) immune response in Burkitt's lymphoma (BL) patients. Anti-MA responses greater than 2.5 logs correlated with a lower frequency of extradural relapse.

The presence of EBV-specific killer T cells in the circulation of acute infectious mononucleosis patients correlated with IgM-antiviral capsid antigen antibody and the absence of anti-EBNA antibody. The T cells could be isolated from tumor tissue and regional lymph nodes but not from the peripheral blood of BL and NPC patients.

Certain epithelial/BL hybrid cells can be infected with EBV. These cells express the HLA antigens of both parental lines but not the C3 receptors. The availability of hybrid cells which lack cell surface markers expressed on one parental line is useful for defining the genetic control and regulatory mechanisms involved in their expression and in studying their function in virus infection.

HSV Studies: Studies on the transcription of HSV DNA in the nuclei of infected cells revealed that only 30% of the messenger RNA species transcribed by an RNA polymerase II-like activity is viral and hybridizable to HSV DNA.

Mouse cells biochemically or morphologically transformed by HSV were found to contain viral DNA fragments ranging in size from 28% of the complete HSV genome down to 3-4% of the genome. The maintenance pattern of the viral DNA did not correlate with the size of the viral DNA fragment. The upper limit of HSV DNA needed for transformation of cells in culture is 3-4% of the virus genome. In one class of transformed cells, no viral DNA was detected after 60 passages of the culture; however, the transformed phenotype persisted. Study of revertant clones from a biochemically transformed cell line clearly indicated that a single copy of the viral thymidine kinase gene was adequate for biochemical transformation. The transforming viral DNA fragment has a base composition like the HSV genome and does not constitute a low G-C viral DNA region. Cross hybridization between HSV-1 DNA and HSV-2 DNA in cells transformed by each virus is very slight, indicating that the DNA in transformed cell clone 139 is not entirely composed of base sequences common to HSV-1 and HSV-2.

Heat inactivation of HSV-1 and HSV-2 was accomplished within 4 min at 56C; only partially heat-inactivated virus (2-3 min at 56C) induced transformation in PRK cells. Studies on transformation by transfection with sheared and fragmented HSV DNAs indicated that transfection is a feasible approach to determine which fragment of virus DNA is responsible for transformation.

In studies on the interaction of herpesviruses with endogenous type C viruses, reactivity of antiserum to mouse leukemia virus p30 protein with HSV-infected mouse cells was shown not to be due to common antigenic determinants in HSV proteins and p30. Cell DNA synthesis is necessary for p30 induction, whereas virus DNA replication is not. This correlates with the observation that high multiplicities of infection with HSV apparently inhibit p30 induction, presumably by increased rates of viral replication which more rapidly shut off host cell DNA synthesis.

Pre-existing nicks and gaps in HSV DNA may be responsible for the heterogeneous sedimentation patterns of untreated HSV DNA in alkaline sucrose gradients. Using exonuclease III digestion of HSV-1 DNA, it was observed that at least one single-stranded end occurs on 25-30% of the full length linear molecules, no snap-back forms were seen, and circular HSV-DNA was seen only at a low frequency.

Studies on the incorporation of fucose into HSV-transformed hamster and human cells and cervical carcinoma cell lines showed a block in the conversion of fucose to fucolipid IV in malignant cell phenotypes. This block was shown to correlate with the cell cycle dependent expression of the early nonstructural polypeptide, VP134.

Significant progress has been made in the purification of HSV-induced proteins and glycoproteins and the preparation of antisera to individual HSV polypeptides. Included among these are serological reagents for and preparations of the nonstructural polypeptides, VP175 and VP143, the major glycoproteins, VP123,

VP 119, VP88, and VP58. VP123 and VP119 were found to possess predominantly type-specific antigenic determinants, i.e., VP123 for HSV-1 and VP119 for HSV-2. Using a competitive radioimmune precipitation test, it was shown that type-specific antibody can be detected in human sera from individuals with primary HSV-1 or HSV-2 infections.

In primary HSV-2 infections, antibody to AG-4, an early HSV-2 structural protein, precedes detection of neutralizing HSV-2 antibody and is transient, disappearing in several weeks after infection. Disease recurrence does not appear to stimulate measurable amounts of AG-4 antibody. The association of this antigen with cancer of the cervix may not be due to tumor specificity of the antigen but rather results from the regulation of AG-4 expression and its availability as an immunogen. Another antigen associated with cervical cancer was designated AG-e. This antigen also appears to be located on the surface of the virion, is not involved in neutralization, and is present in exfoliated cervical tumor cells.

Serum from patients with cervical dysplasia or carcinoma in situ was shown both to modify and suppress the lymphocyte response to two nonspecific mitogens, PHA and Con A. Although responses to purified HSV-1 and HSV-2 vary from patient to patient within the same category, the responses of cervical cancer patients appear to be depressed in comparison with responses of patients with recurrent herpetic lesions.

Small DNA Viruses: Highly specific, characterized probes for the detection of transforming gene(s) of adenoviruses (Ad) were employed in an extensive survey of human tumors. Ad12 and probably all group A adenoviruses were ruled out as etiologic agents for gastro-intestinal tumors. Group C adenoviruses were definitely excluded as the cause of colo-rectal or lung cancers. Group B adenoviruses were not implicated as major causes of lung cancer.

Genome copies of BK papovavirus, which is widely distributed in humans, were detected in five of 12 human tumors and in three of four tumor cell lines. No BK virus DNA sequences were detected in the DNA of normal tissues nor was infectious papovavirus detected in the two BK virus DNA positive tumors tested.

R-MLV, Mo-MLV, and Ki-MLV increase permissivity of NIH-3T3 cells for the polyoma mutant NG-18, indicating the ability of MLV to alter the expression of cellular genes.

Complement fixation, fluorescent microscopy, and electron microscopy studies of Bean epithelial cells infected with human wart virus indicate that virus multiplication occurs only in cells infected at a low passage number. New virus antigen is detected at 4-5 days after infection and reaches a maximum on the 8th to 9th day. Suggestive evidence was obtained that epidermodysplasia verruciformis virus is antigenically distinct from both hand and plantar wart viruses.

Herpesviruses in Nonhuman Primates and Lower Vertebrates: An EBV-like virus was isolated from cell lines established from baboons with lymphoid disease and was shown to have cell transforming activity in vitro. Cells of the

original lines and the transformed cells showed no staining for EBNA. The baboon virus DNA was shown to have 50% homology with EBV DNA from P3HR-1 cells.

An anti-lymphocyte factor produced by a continuous line of herpesvirus saimiri (HVS)-lymphoid cells was shown to be nonsedimentable, resistant to temperatures of 56C but not 80C, nondialyzable, and resistant to DNAse, RNAse, and trypsin. The factor inhibited DNA, RNA, and protein synthesis but did not affect cellular respiration.

Herpesvirus ateles (HVA) was shown to produce a fatal leukoproliferative disease in owl monkeys, New Zealand white rabbits and inbred ACCRB rabbits. No infection or disease was produced in Cebus albrifrons, Macaca fascicularis, or Macaca cyclopis.

Purified HVS DNA was shown to cause malignant lymphoma in Saguinus oedipus and in New Zealand white rabbits.

The MKT-1 lymphoblastoid chicken cell line is a nonproducer of Marek's disease herpesvirus (MDV) as indicated by the inability of virus to replicate when the MKT-1 line is co-cultivated with permissive cells. Electron microscopic examination of several hundred IdU treated MKT-1 cells revealed the presence of both herpesvirus and type C virus, but in no instance were both viruses detected in the same cell. Transformed cells were characterized as T cells and were shown to contain antigens related to both MDV and avian leukosis virus (ALV). Overt tumors were induced in SPF chickens inoculated with viable cells of producer line MSB-1, or the nonproducer line MKT-1, or the transformed WBC-1. MDV was isolated from the feather follicle epithelium of all tumored chickens. Chick embryo fibroblast cultures produce the p19 of ALV for a short time soon after infection with MDV; this observation may correlate with the enhancement of oncornavirus expression in the tumor tissue of chickens inoculated with ALV-free MDV.

BAYLOR COLLEGE OF MEDICINE (NO1-CP5-3256)

Title: Studies on Viruses Related to Cancer

Contractor's Project Director: Dr. Joseph L. Melnick

Project Officer (NCI): Dr. Berge Hampar

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: Work continued on the development of a type-specific test for the measurement of herpes simplex virus (HSV)-1 and HSV-2 antibodies in human sera. Radioimmune precipitation measures type-specific antibody in human serum by using the SDS-PAGE-purified major glycoprotein of HSV-1 and HSV-2. An advantage of this test is that type-specific antibody can be detected without prior adsorption of the serum.

Transformation of hamster and human cells was accomplished using ultraviolet light- and heat-inactivated HSV and HSV temperature-sensitive mutants. Colony formation in soft agar was shown to be a more sensitive method than focus formation in cell monolayers for detecting oncogenically transformed cells which express HSV antigens. HSV-transformed hamster and human cells and cervical carcinoma cell lines were studied for their incorporation of radioisotopically-labeled fucose into fucolipids (FL) III and IV. A block in the incorporation of label into FL IV appears to be correlated with the expression of the malignant phenotype.

HSV-transformed cells were found to express HSV-specific gene products in indirect immunofluorescence tests using antisera prepared against individual HSV-specified polypeptides (VP143 and VP175). In HSV-transformed hamster cells, the expression of an early nonstructural polypeptide, VP134, was shown to be cell-cycle-dependent. The membrane FL block was also found to correlate with the expression of VP143 in transformed hamster and human cells.

The HSV DNA polymerase was purified and characterized. Antiserum neutralizing this activity was prepared in rabbits. The reagent will aid in detection of HSV polymerase activity in transformed cells or in cancer cells obtained from patients.

Some 17 DNA-binding proteins from HSV-infected cells were characterized. A search for these species in transformed cells was begun with initial encouraging results.

Significance to Biomedical Research and the Program of the Institute: Serological and sero-epidemiological data obtained by controlled studies on patients with squamous cell carcinomas suggest that genital and oral strains of herpes simplex virus may be factors in the genesis of these malignancies. Intensive study of the biochemical, genetic, and immunological aspects of the herpesviruses associated with these neoplasms provides information contributing to a determination of the role of these herpesviruses in the development of squamous cell carcinomas in humans.

Proposed Course: To investigate the possibility of causal relationships of herpesviruses types 1 and 2 to human carcinomas, the following objectives are being emphasized by the contractor: (a) detection of HSV gene functions in human carcinomas by complementation and marker rescue studies; (b) search for virus-specific antibodies and antigens in human tissues using purified specific virion and nonvirion antigens of HSV; and (c) insights into cell transformation and genetics by temperature-sensitive and UV mutants of HSV.

Date Contract Initiated: June 27, 1963

Current Annual Level: $349,900

CALIFORNIA, UNIVERSITY OF, IRVINE (NO1-CP5-3560)

Title: Relationship of Herpes Simplex Virus Type 2 to Human Urogenital Cancer

Contractor's Project Director: Dr. David T. Kingsbury

Project Officer (NCI): Dr. George Vande Woude

Objectives: To obtain, by biochemical methods, knowledge of the virus-cell relationship established in transformations induced in experimental systems and to apply sensitive nucleic acid hybridization methods to probe for the presence of specific herpes simplex virus (HSV)-2 nucleic acid in cervical and prostatic carcinomas.

Major Findings: Analysis of a series of 40 independently isolated HSV biochemically or morphologically transformed mouse cells led to the identification of three classes of transformed cells with respect to maintenance of the viral DNA. These cell lines contained viral fragments which ranged in size from 28% of the HSV genome to 3-4% of the genome. The maintenance pattern of the viral DNA did not correlate with the size of the viral DNA fragment but did correlate with the selection criteria applied to the original isolation. It is concluded from these experiments that 3-4% of the genome is the upper limit of HSV DNA required for transformation of cells in culture. In one class of transformed cells (class 3) no detectable viral DNA remains in the cell after 60 or more tissue culture passages; however, the transformed phenotype persists.

An examination of revertants isolated from a line of biochemically transformed cells showed that of five revertant clones selected for ^{3}HTdR or BUdR resistance, four had lost all detectable viral DNA while the fifth, selected for BUdR resistance, retained the entire viral fragment; however, there was a reduction of viral copies per cell from 5 to 1. Three "supertransformed" revertant cell lines contained viral DNA fragments ranging from 12 to 28% of the viral genome. The number of viral DNA fragments per cell ranged from 1 to 5 and clearly indicated that a single copy of the viral thymidine kinase gene is adequate for biochemical transformation. The determination of the base composition of the transforming viral DNA fragment indicated that the transforming DNA has a base composition approximately the same as the HSV genome and did not constitute a low GC viral DNA region. Cross hybridization between HSV-1 transformed cells and HSV-2 DNA is very slight indicating that the DNA found in clone 139 is not entirely composed of the common nucleotide sequence in HSV-1 and HSV-2.

HSV stimulates DNA synthesis in mouse spleen cultures. The newly synthesized DNA is host cell DNA and not of viral origin. The cell type stimulated to undergo DNA synthesis following HSV infection appears to be the bone marrow-derived (B) lymphocytes. HSV stimulates DNA synthesis in normal, macrophage-depleted, and T cell-depleted spleen cultures, but not in thymocyte cultures. In addition, a polyclonal response is observed in HSV-infected spleen cultures. Heat treatment of HSV destroys its ability to stimulate DNA synthesis. Inactivation of HSV by ultraviolet irradiation prior to addition to cultures does not affect the induction of DNA synthesis. These findings imply that HSV transforms the B lymphocytes. This system appears to provide an assay for the study of cell transformation by HSV. The system may also be used for the study of host cell control of the expression of the viral genome.

Five additional cervical carcinomas have been examined. None of these tumors showed any evidences of HSV genetic information. However, these results are subject to the technical limitation imposed by the HSV hybridizations at present. A modification of the present system is being developed and should increase the sensitivity of the procedure manyfold.

Significance to Biomedical Research and the Program of the Institute: Sero-epidemiological studies have shown an association between HSV-2 infection and human cercival carcinoma. To further define the role, if any, of this virus in oncogenesis, these studies are to determine whether HSV-2 genetic information can be demonstrated within cervical tumor cells, and, further, to develop a better understanding of the nature of the interaction between the virus and cells which leads to their transformation experimentally.

Proposed Course: Continue studies, by molecular biology, on HSV DNA in relation to neoplastic transformation of human and animal cells.

Date Contract Initiated: May 16, 1975

Current Annual Level: $100,000

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP7-1009)

Title: Interaction of Human Papovaviruses BK and JC and Simian Virus 40 with Human Vascular Endothelial Cells

Contractor's Project Director: Dr. George C. Fareed

Project Officer (NCI): Dr. George Khoury

Major Findings: This project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute:

Papovaviruses have been shown to produce cancers in experimental animals, but the relationship of these viruses to human cancer has not been defined. The isolation of human papovavirus strains from urine, brain tissue, and certain tumors necessitates investigation of their transforming activity in human cells and association with human disease. This project provides the opportunity to use a human diploid cell system to study the molecular interactions of viral genomic sequences with human cell DNA with respect to cell transformation and to acquire a well-characterized system for study of the relationships of papovaviruses to human neoplasia.

Proposed Course: The project will continue without change.

Date Contract Initiated: January 15, 1977

Current Annual Level: $57,300

EMORY UNIVERSITY (NO1-CP4-3393)

Title: Cellular Immunity Studies to Herpes Simplex Virus-Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. André Nahmias

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: The major objectives of this contract are: (1) to provide the necessary immunological infrastructure required to evaluate any prospective herpes simplex virus (HSV) vaccine for human use; (2) to obtain evidence corroborating the hypothesis that HSV-2 plays an etiological role in human cervical neoplasia; and (3) to provide a better understanding of the host factors involved in primary and secondary HSV infection.

Major Findings: Serum from patients with cervical dysplasia or carcinoma in situ (CIS) has been shown to both modify and suppress the lymphocyte response to two nonspecific mitogens, PHA and Con A. Studies are being carried out to identify the active agent. HSV-1 and HSV-2 have been purified,

and are being used as specific antigens. The responses to these antigens vary between individuals in the same patient category; however, the responses of the cervical cancer patients appear to be depressed when compared with the responses of recurrent herpes patients. A suppressive effect of autologous serum on the response to HSV antigens has not yet been demonstrated. The effect of temperature, which affects the magnitude of lymphocyte transformation, is also being investigated in an attempt to identify specific defects in the immune response of study patients. In this context, patients with CIS have significantly fewer lymphocytes capable of forming E rosettes than normal women. The inhibition of E rosette formation is being measured by anti-lymphocyte serum, as this assay has been shown in other systems to discriminate between patients with malignancies and those with benign tumors.

A new activity of a soluble lymphocyte product has been demonstrated. Lymphocytes from sensitized donors, in the presence of the sensitizing antigen, release a soluble factor which stimulates the metabolism of the hexose monophosphate shunt in human polymorphonuclear leukocytes (PML). The reaction is specific for the sensitizing antigen, and the mediator is released from the sensitized lymphocyte upon contact with the antigen. This assay is being refined, and is being applied to the study patients.

Antibody-dependent lymphocyte cytotoxicity (ADLC) has been investigated at both basic and clinical levels. The lymphocytes which mediate ADLC have been shown to be neither T cells nor B cells, and have the characteristics of "K" cells. Additional leukocyte populations which can act as effector cells in ADLC have been defined. PML can kill HSV-infected target cells in the presence of HSV antibodies and the reaction has different parameters than those mediated by "K" cells. Purified eosinophils from some patients also demonstrate these effects. Similarly, an adherent mononuclear effector cell population with characteristics of monocyte-macrophages can mediate ADLC but shows differences in reaction parameters from "K" cell killing. The adherent mononuclear cell reaction can be inhibited by incubation of the effector cells with particulate matter, whereas the "K" cell reaction cannot.

No differences were observed in titers of lymphocyte-dependent antibodies cytotoxic to HSV-infected target cells between cancer patients and controls; hence, future efforts will be directed towards an analysis of the relative importance of the various effector cell types in neoplastic disease. Investigations of direct lymphocyte cytotoxicity, and complement-dependent antibody cytotoxicity have not been productive. By various serological methods further evidence was obtained for cross-reacting antigens between HSV-2 and cervical cancer. In addition, newly developed assays are being used to detect antigen-antibody complexes in the serum of patients, as well as on cervical cancer cells.

Significance to Biomedical Research and the Program of the Institute: The evaluation of cell-mediated immune responses to HSV-2 in patients with cervical carcinoma may add further information for determining whether there is an etiological link between the virus and the neoplastic disease and on host factors involved in HSV-2 infection and disease.

Proposed Course: Studies will be completed by the end of the current contract year.

Date Contract Initiated: June 28, 1974

Current Annual Level: $70,000

HARVARD UNIVERSITY (NO1-CP4-3299)

Title: Investigation of Cellular, Exogenous and Endogenous RNA Viral Factors in Replication and Transformation by Polyoma Virus

Contractor's Project Director: Dr. Thomas L. Benjamin

Project Officer (NCI): Dr. Robert Goldberg

Objectives: Investigate the relationship between cells transformed by DNA and RNA tumor viruses, specifically, to test the ability of various cell lines, transformed or infected by various DNA or RNA tumor viruses, to complement the growth defect of the polyoma virus mutant, NG-18.

Major Findings: Major effort continues on the question of the murine leukemia virus (MLV) effect in converting NIH-3T3 cells from nonpermissive to permissive for hr-t mutants of polyoma. Three temperature sensitive mutants of Rauscher-MLV are used in this approach. Chronically infected and virus-producing clones of NIH-3T3 cells have been sought with mutants ts-25, ts-26, and ts-29, and with wild type Rauscher-MLV. The latter virus, along with Moloney-MLV and Kirsten-MLV, has already been shown to increase the permissivity of NIH-3T3 cells. Several clones initially positive for each virus were isolated and frozen. On thawing, it was found that most clones either lost virus-producing ability, or produced only small amounts of virus at 32.5C. The reason for the lack of stability on freezing and thawing is not known. To circumvent this problem, one wild type producer and one producer of ts-29 have been isolated and maintained continuously at 32.5C without freezing. With these and normal NIH-3T3 cells as a control, permissivity experiments are being carried out using NG-18 and wild type polyoma virus.

Significance to Biomedical Research and the Program of the Institute: The study deals with an extension and confirmation of earlier observations which suggested that similar cellular functions were expressed following transformation of cells by either RNA or DNA viruses. This is important since it may help to define the intracellular events essential for cell transformation.

Proposed Course: This contract was terminated on June 30, 1977.

Date Contract Initiated: April 1, 1974

Current Annual Level: $89,035 (for nine months)

HARVARD UNIVERSITY (NO1-CP3-3390)

Title: Oncogenic Herpesviruses in Primates

Contractor's Project Director: Dr. T. C. Jones

Project Officer (NCI): Dr. Dharam Ablashi

Objectives: To study oncogenic herpesviruses in primates.

Major Findings: Herpesvirus ateles (HVA) has been shown to induce a fatal leukoproliferative disease in owl monkeys. Earlier studies did not indicate that owl monkeys were susceptible to HVA. This suggests that the response of owl monkeys to HVA may be variable as it is with Herpesvirus saimiri (HVS). HVA was shown to cause malignant lymphoma in New Zealand white rabbits and inbred ACCRB rabbits. No disease was associated with the inoculation of HVA in Cebus albifrons, Macaca fascicularis or M. cyclopis.

It was shown that purified HVS DNA will cause malignant lymphoma in Saguinus oedipus and New Zealand white rabbits.

HVS has not been shown to cause disease in Cercopithecus aethiops; however, experimental inoculation does elicit an antibody response and increases the mitotic index to phytohemagglutinin in lymphocytes. No response was seen in M. fasicularis or M. cyclopis inoculated with HVS.

The effect of HVS on owl monkeys with each of three of the seven known karyotypes identified in this species has been shown to be roughly equivalent.

Significance to Biomedical Research and the Program of the Institute: Several different herpesviruses induce lymphoid neoplasms in animals, and some members of this DNA virus group are suspected to be oncogenic in humans. HVS is of particular interest because it produces T-cell lymphoma in two dissimilar animal species and provides a system for the investigation of viral, cellular, and host factors which influence the development of overt disease. This will contribute to an understanding of virus-host interactions which bear upon the development of neoplasia in humans.

Proposed Course: This project was terminated on September 30, 1977.

Date Contract Initiated: June 26, 1972

Current Annual Level: $92,000

HEBREW UNIVERSITY (NO1-CP3-3310)

Title: Studies on Herpesvirus and its Role in Human Cancer

Contractor's Project Director: Dr. Yechiel Becker

Project Officer (NCI): Dr. Robert Goldberg

Objectives: Investigate the transcription of herpes simplex virus DNA and Epstein-Barr virus DNA.

Major Findings: Studies on the transcription of herpes simplex virus (HSV) DNA in the nuclei of infected cells revealed that only part (about 30%) of the messenger RNA species that is transcribed by an RNA polymerase II-like activity is viral and hybridizable to HSV DNA. Separation of the poly A-containing virus mRNA species from cellular RNA was achieved by the use of a HSV DNA-Sepharose column. The use of an α-amanitin resistant mutant cell line revealed that the cellular RNA polymerase II is responsible for the transcription of HSV DNA. HSV replicated in α-amanitin-resistant cells in the presence of α-amanitin and amphotericin B indicating that a virus specific RNA polymerase is not required. Chromatography of the RNA polymerases isolated from infected nuclei on DEAE-Sephadex and dsDNA-cellulose columns revealed one peak of RNA polymerase II activity. In uninfected cells a number of peaks of RNA polymerase II activity were obtained. Conditions were developed to study RNA synthesis in isolated nuclei *in vitro* to define the requirements for transcription by the RNA polymerases. Inhibition of the RNA polymerase activity by α-amanitin resulted in the stimulation of DNA biosynthesis. RNA polymerases were also isolated from lymphoblastoid cells and were found to differ from the RNA polymerases in growing BSC-1 cells. It is not yet known which RNA polymerase in lymphoblastoid cells transcribes Epstein-Barr virus DNA. Studies were begun on DNA synthesis in isolated nuclei and on the molecular forms of HSV DNA. Various molecular forms of HSV DNA that occur in infected nuclei (e.g. replicative intermediates) have been observed and investigated. HSV DNA transcription complexes have not yet been found.

Significance to Biomedical Research and the Program of the Institute: The studies on the molecular processes involved in the regulation of the herpesvirus DNA genomes in permissive and transformed cells will provide basic information on the nature of the herpesvirus induced antigens and their role in the ability of the tumorigenic cells to proliferate and develop as tumors in humans.

Proposed Course: This contract was terminated on July 31, 1977, and the funds transferred to the CREG program.

Date Contract Initiated: June 29, 1973

Current Annual Level: $74,656

ILLINOIS, UNIVERSITY OF (NO1-CP4-3318)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. Robert Goldberg

Objectives: Characterization of the human papovaviruses, especially BK virus.

Major Findings: Deletion mutant d1 884 of SV40 was shown to be highly impaired in its transformation capability but able to replicate in the permissive host.

The deletion corresponds to the mapped location of NG-18, a nontransforming mutant of polyoma virus. The virus mutant, dl 884, will be valuable in the study of the transformation maintenance gene of SV40.

BK virus was shown to require a longer growth period (40 hr) in HEK cells than SV40, polyoma, or humanderived SV40 variants (20 hr).

The DNA of four different papovavirus isolates (DW, GS, MG, RF) from human urines isolated in England, South Africa, and the United States were compared with BK DNA for homogeneity of molecule population, genome size, and, by use of restriction endonucleases, the number and distribution of recognition sites on the viral genomes. Supercoiled molecules comprised 60-70% of the DNA's of the isolates with the remainder as nicked circular molecules. The genome size of BK, GS, and DW is slightly larger than the genomes of RF and MG. The DNA of each of the five isolates has one recognition site for R-EcoRI, and none for R-HaeII. The DNA's of RF, GS, DW, and MG were shown to be more than 90% homologous to the DNA of BK.

Genome copies of BK virus were detected in five of 12 human tumors examined and in three of four tumor cell lines. No BK DNA sequences were detected in DNA of normal tissues nor was infectious virus detected in the two BK DNA positive tumors tested.

Significance to Biomedical Research and the Program of the Institute: Studies on small icosahedral DNA viruses such as SV40 and polyoma have contributed an impressive body of information in recent years on the interactions between these viruses and cells. The genetic analysis of the genome of these viruses, by the use of mutants, may provide a major advance in the knowledge of the mechanism of cell transformation at the molecular level. These studies have recently become of great importance with the isolation of human papovaviruses, such as BK and JC viruses.

Proposed Course: Greater emphasis will be placed on the development of the BK virus biological system and determination of the presence or absence of the BK virus genome in human tumors.

Date Contract Initiated: December 9, 1971

Current Annual Level: $379,836

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3345)

Title: Herpesvirus Antigens and Virions in Neoplastic Cells from Cervical Carcinoma

Contractor's Project Director: Dr. Laure Aurelian

Project Officer (NCI): Dr. George Vande Woude

Objectives: Identification of herpes simplex virus type 2 (HSV-2) antigens and virions in neoplastic human cells to develop evidence for or against HSV-2 as a factor in the etiology of squamous cell carcinoma of the human uterine cervix.

Major Findings: AG-4 and ICP 10 proteins are located in the lysates of nuclei from HSV-2 infected cells together with viral proteins 5, 6, 10, 13, 16 and 20. ICP 10 and AG-4 are not located in the capsids but probably in the viral envelope.

Antibody to AG-4 is found in women with primary HSV-2 infections, further pointing to the virus specificity of AG-4 antigen. AG-4 antibody is transient and disappears within 20-160 days post infection. Most women with recurrent disease are AG-4 negative, most probably due to the low levels of AG-4 available as immunogen and the 19S nature of the AG-4 antibody.

ICP 10 was purified by serial high resolution SDS-acrylamide gel electrophoresis. Antiserum to ICP 10, prepared in rabbits, fixes complement with AG-4. In anticomplement immunofluorescence (ACIF), it stains cells infected with HSV-2 in presence of cycloheximide. ICP 10 is the major protein synthesized under these conditions, and AG-4 antigen is increased in these cells.

Ag-e is a major, type-common HSV antigen located on the surface of the virion but not involved in virus neutralization. It is present in exfoliated cervical tumor cells. It probably represents a virion subunit consisting of a number of proteins.

Using the indirect radioimmunoprecipitation technique, patients' sera preferentially precipitate a number of viral proteins which are also precipitated by antiserum to AG-e, suggesting that cancer patients may have higher levels of antibody to AG-e than do controls.

Significance to Biomedical Research and the Program of the Institute: Considerable data has been acquired demonstrating an association between HSV-2 infection and carcinoma of the uterine cervix. In humans, it is extremely difficult to show conclusively that virus associated with neoplasms is a factor in oncogenesis and not simply a passenger. Studies conducted under this project are expected to provide some of the data required to reach a decision regarding the role of this virus in oncogenesis.

Proposed Course: Studies were completed at the end of the current contract year.

Date Contract Initiated: May 5, 1971

Current Annual Level: $88,050

JOHNS HOPKINS UNIVERSITY (NO1-CP4-3266)

Title: Studies of New Papovaviruses Isolated from Man

Contractor's Project Director: Dr. Richard T. Johnson

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Characterization of papovaviruses isolated from humans and determination of their presence in human sera, urine and human cerebral neoplasms.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: Papovaviruses are known to cause tumors in animals and to transform cells in culture. Since there is serological evidence that human infection by certain papovaviruses is rather common, it is important to assess the role of these viruses in human cancer.

Proposed Course: This project was terminated on January 31, 1977.

Date Contract Initiated: February 1, 1974

Current Annual Level: None

JOHNS HOPKINS UNIVERSITY (NO1-CP4-3330)

Title: Studies on Cellular Immunity to Herpes Simplex Virus-Associated Antigens in Cancer Patients and Controls

Contractor's Project Director: Dr. Laure Aurelian

Project Officer (NCI): Dr. Steven R. Tronick

Objectives: The objectives of this contract are (1) to establish whether cell-mediated immunity (CMI) to herpes simplex virus type 2 (HSV-2) antigens can be demonstrated in selected cancer patients; (2) to identify and characterize the antigens responsible for eliciting the cellular immune responses; (3) to correlate CMI responses with serological assays designed to detect humoral antibody to HSV-2 antigens; and (4) to relate the results of these studies to the clinical status of patients and controls.

Major Findings: Exfoliated anaplastic cells obtained from cervical carcinoma patients were shown by indirect immunofluorescence to contain a major, type-common HSV antigen. The antigen, designated AG-e, was obtained by calcium phosphate chromatography of solubilized HSV-2 infected cells and was used to prepare specific antisera. Fluorescent staining of exfoliated cervical tumor cells by anti-AG-e was detected in 16 of 21 patients. Among these 16 patients, an average of 18% of anaplastic cells were found to be AG-e positive. In addition, 85-100% of cells with characteristic herpetic cytopathology obtained

from patients diagnosed as having herpetic cervicitis were found to stain with this antiserum.

By gel immunodiffusion, antisera to AG-e prepared from HSV-1 or HSV-2 infected cells was found to produce a single precipitin band of identity when reacted against soluble antigenic mixtures from HSV-2 infected cells. Preliminary characterization of AG-e using neutralization blocking techniques and an antiglobulin enhancement neutralization assay suggested that the antigen is located on the envelope of infectious virions but does not play a significant role in virus neutralization.

By a leukocyte inhibitory factor (LIF) assay, 70% of patients with cervical anaplasia and 100% of patients with recurrent HSV disease were found to respond to AG-e. Significantly, AG-e also represents a major viral antigen in exfoliated cells displaying herpetic cytopathology collected from patients with herpetic cervicitis. In contrast to the high frequency of positive LIF responses in the cervix cancer patients, positive responses to this antigen were seen in only 13% of matched control women without cancer and 16% of patients with neoplasia at sites other than the cervix. The longitudinal study of patients with latent infections experiencing recrudescences, as well as the temporal study of HSV-2 infected guinea pigs, indicated that AG-e induced LIF is a transient, "antigen-driven" phenomenon, possibly mediated by thymus-derived (T) lymphocytes.

Significance to Biomedical Research and the Program of the Institute: The evaluation of CMI responses to HSV-2 in patients with cervical carcinoma may add further information for determining whether there is an etiological link between the virus and the neoplastic disease and on host factors involved in HSV-2 infection and disease.

Proposed Course: Studies were completed at the end of the current contract period, June 30, 1977.

Date Contract Initiated: June 28, 1974

Current Annual Level: $150,000

KAROLINSKA INSTITUTE (NO1-CP3-3316)

Title: Studies on the Significance of Herpes-Type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officer (NCI): Dr. Berge Hampar

Objectives: To elucidate Epstein-Barr virus (EBV)-cell-host interactions, mechanisms of cell-mediated anti-tumor immune reactions, and regulation of type C virus expression in defined systems.

Major Findings: More than 90% pure EBV-DNA circles were isolated from the cellular DNA of EBV-carrying lines. A clear difference in circle size has

been found between certain IM and BL derived lines. It is not yet clear whether this is a virus strain difference or a disease related difference.

In some early antigen positive (EA^+) cell lines which synthesize EBV DNA, the production of virus capsid antigen (VCA) may be blocked.

All cells which carry EBV contain EB nuclear antigen, EBNA. EBNA was purified, concentrated and characterized. Its antibody binding ability is dependent on the presence of DNA, but the source of DNA is not specific. Elution characteristics and immunological evidence suggest EBNA-subtype differences. Brilliant EBNA staining could be created in EBNA-negative human and animal cells by acid-extraction of the nuclei, and subsequent addition of semi-purified EBNA. This is the first direct proof that the complement-fixing (S) antigen and EBNA are identical.

Virus producer cells could be separated from nonproducer cells by retention on IgG columns, after selective antibody coating, or on EBV-receptor columns without antibody coating. The column-method was useful for blocking studies concerning the relationships between the EBV and the complement receptor, and for demonstrating EBV receptor subtype differences between baboon and human cells.

An *in situ* radioimmunoassay (RIA) developed for EA and VCA at the single cell level, revealed that several lines, previously considered as nonproducers, in fact did produce viral antigens in a small proportion of the cells. EA induction experiments with ultraviolet-inactivated virus on EBV genome negative cell lines and the derived, EBV converted sublines confirmed the previous postulate that the resident EBV genome participates in EA induction, following infection with P3HR-1 virus.

EBV cycle related studies on new cell hybrids confirmed that the complex regulatory patterns of the hybrids are not haphazard, but reproducible for a given cell combination. It was also shown that while EA production is a prerequisite of VCA production, the latter is regulated by partly independent controls.

In the TA3Ha/Daudi cell hybrid series, chromosome 21 is most likely responsible for EBV DNA and EBNA maintenance. Suggestive evidence implicating chromosome 21 has also come from an independent series of nasopharyngeal carcinoma (NPC) biopsy/mouse fibroblast hybrids. The latter series includes the first definite NPC cell derivative *in vitro*, opening the way for direct studies on these previously unavailable cells in culture.

Polyclonal IgM synthesis was induced by EBV in freshly infected human B-lymphocytes, suggesting that the virus acts like a true polyclonal B-cell activator.

EBV conversion of negative cell lines to linescontaining EBV DNA and EBNA increased complement consumption, increased tolerance to saturation conditions, decreased serum dependence, induced independence of dialysable serum factors and clonability on soft agar, decreased capping and increased agglutinability with con A, decreased surface IgM redistribution, and increased fibrinolytic activity.

BCG selectively stimulated the anti-membrane antigen (MA) response of Burkitt's lymphoma (BL) patients, in contrast to other EBV or nonEBV responses. Anti-MA increases by more than 2.5 logs were correlated with a lower frequency of extradural relapse. Studies on the occurrence of EBV genomes in human malignancies from Uganda confirmed the 97% virus association with African BL from Kenya, did not demonstrate EBV genomes in African nonBL lymphomas, and showed a regular and exclusive association with anaplastic NPC regardless of geographic origin, provided the biopsy used for molecular hybridization was critically shown to contain viable tumor tissue of the proper histology. In nonafrican BL, the incidence of EBV genome carrying tumors is between 8 and 17%.

The presence of EBV-specific killer T-cells in the circulation of acute infectious mononucleosis (IM) patients correlated with the presence of IgM-anti-VCA antibodies and the absence of anti-EBNA. EBV-specific T-cells could be isolated, although more irregularly, from the tumor tissue and the regional lymph nodes of BL and NPC patients and not from the peripheral blood.

In monolayer grown human tumor cell systems, attention was given to the selective removal of nonspecific cytotoxic (NSC) cells located in a nonT, nonB fraction. Purified T-cell fractions lacking NSC could be obtained by multistep fractionation.

In type C virus studies in murine models, findings include an individually distinct antigenic specificity in polyoma induced tumors, reciprocal modulation of various tumor antigens, demonstration of different sensitivities to T versus nonT killer mechanisms in tumors induced by related viruses but in different tissues, studies of the "natural killer" phenomenon, biochemical characterization of the Moloney virus induced cell surface antigen and demonstration of its distinctness from known virion proteins such as gp70, p30, p15, p15(E), p12, and p10.

Significance to Biomedical Research and the Program of the Institute: Investigations under this project are directed to two areas of importance to overall Program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EBV and herpes simplex virus type 2 are associated with human neoplasms requires intensive study to provide a better understanding of the host-virus relationship for this group of agents. Data acquired under this project contribute to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, using defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will continue without change.

Date Contract Initiated: April 9, 1968

Current Annual Level: $176,000

LIFE SCIENCES, INC. (NO1-CP3-3205)

Title: Studies on Marek's Disease as a Model for Herpesvirus-Associated Oncogenesis

Contractor's Project Director: Dr. Meihan Nonoyama

Project Officer (NCI): Dr. George Vande Woude

Objectives: To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease and the elucidation of the mechanisms of interaction between herpes and RNA viruses in tumorigenesis using specific pathogen-free avian hosts.

Major Findings: The initial studies were concerned with the propagation and characterization of a cell-free standard Marek's disease herpesvirus (MDV) derived from LSI-SPF chickens. As a possible large scale source for MDV DNA, infected chicken embryo fibroblast (CEF) cultures were found to produce 0.5 µg of MDV DNA per roller bottle. The MKT-1 lymphoblastoid cell line was a nonproducer of MDV as indicated by the inability of the virus to replicate when MKT-1 line was co-cultivated with permissive cells. Electron microscopic examination of several hundred IUD treated MKT-1 cells revealed the presence of both herpesvirus and type C virus, but in no instance were both viruses detected in the same cell. Cells of a transformed culture, WBC-1, derived from the buffy coat blood cells of an LSI-SPF chicken inoculated with MKT-1 cells, were characterized as T-cells and were shown to contain antigens related to both MDV and avian leukosis virus (ALV). Inoculation of LSI-SPF chickens with viable cells of the virus producer cell line MSB-1, the nonproducer cell line MKT-1, or the transformed cell culture WBC-1 produced overt tumors and mortality, although the levels of these responses varied with the type of cells inoculated. WBC-1 cells, which showed the highest level of ALV expression in tissue culture, produced the lowest incidence of tumors in chickens. MDV, identified by neutralization, was isolated from the feather follicle epithelium (FFE) of chickens of all three groups inoculated with the lymphoblastoid cell cultures.

The oncogenic potential of MKT-1 cells was destroyed by freezing and thawing, as evidenced by the lack of tumor development in LSI-SPF chickens inoculated with the cells killed by this procedure. Karyotypic analyses of transformed cells of chickens inoculated with MKT-1 indicated that the transformation is associated with a chromosome abnormality in the largest No. 1 chromosome pair. Preliminary results indicated that supernatant fluids from the nonproducer cells suppress expression of MDV antigen in producer lymphoblastoid cell lines. CEF cultures evidently produce the p19 polypeptide of ALV for a short time early after infection with MDV. These results may correlate with the previously reported enhancement of oncornavirus expression in the tumor tissue of chickens inoculated with ALV-free MDV. Preliminary results indicate occurrence of an antibody to unique antigen(s) in the nonproducer lymphoid cell line which may influence cell-mediated immunity or afford protection against Marek's disease. A correlation between factors which block cell-mediated immunity and the initiation of neoplastic lesions characteristic of Marek's disease has been observed.

Significance to Biomedical Research and the Program of the Institute: In comparison to the RNA tumor viruses, comparatively little is known concerning the role of herpesviruses in oncogenesis or their interaction with endogenous RNA tumor viruses. Certain herpesviruses have been implicated in the etiology of carcinoma, lymphoma and leukemia in different species of animals and other viruses of this group have been shown to be strongly associated with neoplasia in man. This project provides the opportunity to acquire information on one herpesvirus in relation to a malignant disease in its natural host which may aid in understanding the role of herpesviruses in oncogenic processes in man.

Proposed Course: Studies on the interaction between MDV and other viruses as this relates to the disease process will be continued with particular emphasis on the molecular virology of the interaction between MDV and avian leukosis viruses.

Date Contract Initiated: November 1, 1968

Current Annual Level: $492,440

LITTON-BIONETICS, INC. (NO1-CP5-3509)

Title: Investigations of Suspected Oncogenic Viruses in Nonhuman Primates

Contractor's Project Director: Dr. Harvey Rabin

Project Officer (NCI): Dr. Berge Hampar

Objectives: To investigate host-virus relationships established by viruses known or suspected to induce neoplasia in animals or humans using nonhuman primates of different species and to determine host immune responses following virus infection, correlating these with viral reproduction and pathological processes.

Major Findings: The development of leukemia in owl monkeys infected with Herpesvirus saimiri (HVS) did not impair the ability of the peripheral blood lymphocytes (PBL) of the animals to act as effector cells in the antibody dependent cytotoxicity assay. In contrast to infected but clinically normal owl monkeys, diseased animals showed a loss of responsiveness by lymphocytes to PHA and con A.

A soluble factor, produced by a line of HVS lymphoid cells, blocked response of normal lymphocytes to PHA and inhibited DNA synthesis by several cell types *in vitro*. This factor is not RNA, DNA, protein, or interferon.

Fibrinolytic activity was demonstrable in 3 of 4 mormoset HVS lines, 3 marmoset and 1 owl monkey Epstein-Barr virus (EBV) lines, and 4 of 11 normal peripheral blood lymphocyte lines. A nononcogenic persistent infection with development of antibody to early and late antigens of HVS occurs in young adult capuchin monkeys.

In collaboration with Soviet investigators, three herpesviruses were isolated from diseased baboons from Sukhumi, U.S.S.R. One was EBV-like, the second, foamy virus-like, and the third, typical of cytomegalovirus.

Significance to Biomedical Research and the Program of the Institute: Certain herpesviruses induce lymphoma and leukemia in nonhuman primates. Not all members of a susceptible species develop overt disease despite persistent infection. The comparative study of host immune responses in the course of the disease process and in resistant animals is expected to contribute to a better understanding of host factors in relation to the development of overt neoplasia.

Proposed Course: This project was terminated on June 30, 1977.

Date Contract Initiated: September 1, 1974

Current Annual Level: None

MELOY LABORATORIES, INC. (NO1-NS2-2306)

Title: Oncogenic Potential of Herpesviruses in Primates

Contractor's Project Director: Dr. D. L. Sly

Project Officers (NCI): Mr. J. T. Lewin
(NINCDS): Dr. W. T. London

Objectives: To evaluate herpes simplex virus type 2 (HSV-2) as a potential agent of cervical carcinoma using Cebus monkeys.

Major Findings: This is a collaborative project between NCI and NINCDS. The colony contains 430 Cebus appella monkeys consisting of 305 females and 125 males harem-aged. Females are inoculated every nine months; 231 receive HSV-2; 64, control medium; and 10, 10% salicylic acid. Anaplasia of a mild nature has been detected in cervical smears from 89 virus recipients, 22 controls, and 3 salicylic acid recipients. Persistence (four or more positive smears) has occurred in 16 virus recipients and two salicylic acid recipients. About 78% of the colposcopic and Pap smear evaluations are in agreement.

Significance to Biomedical Research and the Program of the Institute: HSV-2 has been shown to be associated with invasive cervical carcinoma in humans. This project was initiated to determine whether repeated infections in a nonhuman primate that responds to infection by this virus in a manner similar to humans would result in cervical malignancy. The data acquired in this and related projects is expected to provide a better understanding of the nature of the relationship observed between this virus and cervical cancer.

Proposed Course: This project is expected to continue for one more year.

Date Contract Initiated: March 30, 1972

Current Annual Level: $150,000

NORTH CAROLINA, UNIVERSITY OF (NO1-CP3-3336)

Title: Molecular Studies on Herpes-Type Viruses of Potential Oncogenicity

Contract's Project Director: Dr. Joseph Pagano

Project Officer (NCI): Dr. Berge Hampar

Objectives: To define, at the molecular level, the virus-cell relationships of herpes simplex virus type 2 to human cervical carcinoma and of Epstein-Barr virus to Burkitt's lymphoma and nasopharyngeal carcinoma.

Major Findings: This contract was extended for administrative purposes.

Significance to Biomedical Research and the Program of the Institute: The studies accomplished verify the close relationship between Epstein-Barr virus and cells in Burkitt's lymphoma biopsies. The refinement by the contractor of molecular methods provided useful tools in the study of viral associations with tumor cells where repression of virus genetic expression masks the presence of infection.

Proposed Course: This project terminated on November 30, 1976.

Date Contract Initiated: April 25, 1972

Current Annual Level: None

PENNSYLVANIA STATE UNIVERSITY (NO1-CP5-3516)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officer (NCI): Dr. B. Gerwin

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: Quantitative transformation studies with herpes simplex viruses (HSV) have been continued and the following observations have been made. Ultraviolet-light inactivation data are not useful as identification criteria in distinguishing among isolates of HSV. Heat inactivation of HSV-1 and HSV-2 was accomplished within four minutes at 56C, transformants were obtained at 2 to 3 minutes exposure to 56C but not following exposure of virus for 4 minutes or longer, thus providing an excellent method for destruction of transforming activity. Preliminary results suggest that transfection is a feasible approach to determine which fragment of virus DNA is responsible for transformation.

Studies on the interaction of herpesviruses with other viruses has continued. Murine leukemia virus p30 protein was detected with specific antiserum in mouse cells after infection with transforming HSV. At a high multiplicity of infection

of cell cultures with HSV, no p30 is induced even in the presence of HSV antigen synthesis. The reaction is specific for p30 and is not due to reactivity with herpesvirus-specified proteins or virus-damaged infected cells. In addition, it would appear that cell DNA synthesis is necessary for p30 induction while virus DNA replication is not.

Further tests were carried out to examine the oncogenic potential of different sublines of cytomegalovirus (CMV)-transformed cells. Anti-complement immunofluorescence (ACIF) tests were carried out to determine the reactivity of different human sera with the nuclear antigens of the transformed cells. Hamsters were immunized with CMV-transformed human cells and their sera were tested for antibody reactivity in CMV neutralization and immunofluorescence (IF) tests. DNA-DNA hybridization studies were made to detect CMV genome in various tumor lines originated from a CMV-transformed human embryo lung fibroblast cell line in which transformed cells developed at passage 24 after inoculation with CMV. EBNA-like nuclear antigens could be detected in CMV-Mj-HEL-1 cells with some CMV-immune human sera by the ACIF technique. CMV-immune human sera, screened for reactivity with EBNA-like nuclear antigens of CMV-transformed human cells, stained nuclear antigens in 6-80% of the cells.

It has been demonstrated that there are pre-existing nicks and gaps in HSV DNA and that these are primarily responsible for the heterogeneous sedimentation pattern of untreated HSV DNA on an alkaline sucrose gradient. Preliminary studies are underway to characterize the ends of HSV DNA. A procedure for exonuclease III digestion of HSV-1 DNA sufficient to reveal the terminally repetitious ends as single-stranded has been worked out. At least one single-stranded end is seen on 25-30% of full length linear molecules. No snap-back forms have been observed and circular DNA has been observed at low frequency.

HSV DNA has been isolated from a Hirt supernatant. Recovery of virus DNA by this procedure is approximately 72%, and the yield is about 10 μg of HSV DNA per 10^7 cells. This amount is about 50 times greater than that obtained by isolating DNA from intact virions grown in the same number of cells. Preliminary characterization of varicella-zoster virus (VZV) DNA is now underway. It was found that the DNA of VZV is selectively released into the Hirt supernatant and sufficient quantities of VZV DNA are now available.

Significance to Biomedical Research and the Program of the Institute: The observations made under this project indicate that some strains of herpesviruses commonly afflicting humans may express oncogenic properties under certain conditions. Better assay procedures for transforming potential may provide a means to assess the significance of virus strain differences within populations leading to a better understanding of the nature of the association of HSV-2 with human cancer.

Proposed Course: Investigations on herpesviruses in relation to cancer will continue.

Date Contract Initiated: October 27, 1969

Current Annual Level: $447,000

ST. LOUIS UNIVERSITY (NO1-CP4-3359)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. George Vande Woude

Objectives: This research program is aimed to achieve increased understanding of the mechanism of cell transformation by DNA tumor viruses and to apply new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer.

Major Findings: As a result of studies on methodology to detect viral transforming gene(s) (TG) in tumors, a microscale procedure was developed to label adenovirus (Ad) DNA and DNA restriction fragments to 1-2 x 10^8 cpm/μg; probes are uniformly labeled and hybridize faithfully. Ad12 (highly oncogenic group A)-transformed HE C19 cells have a unique pattern of integrated viral genes, as do Ad12 tumors. With in vitro labeled HindIII-G as probe, 5RK cells (a rat line transformed by fragment HindIII-G) were found to contain 70% of the G-fragment; 293-31 cells (a human cell transformed by sheared Ad5 DNA) contained 80-90% of HindIII-G. Thus the HindIII-G probe could detect, if present, group C Ad TG in human tumors.

Study of the distribution of group A and C TG among 31 human adenoviruses demonstrated that group C Ads (Ad1, 2, 5, 6) TG (HindIII-G) are 97-100% homologous to Ads within group C, and nonhomologous to other Ads. Ad12 (Huie) transforming EcoRI-C fragment is 53-68% homologous to other group A Ads, and nonhomologous to other Ads. Group B Ads (Ad3, 7, 11, 14, 16, 21) are 85-100% homologous. Fifteen group D Ads (Ad8-10, 13, 15, 19, 20, 22-24, 26-30) are 90-100% homologous. Ad4 is 20% homologous to other Ads (group E). Ad25 (group G) and possibly Ad17 (group F) are 25% homologous to other Ads. Thus analysis of tumors with 6 or 7 Ad probes can test a cancer etiology for 31 Ad types.

Analyses of human tumors were conducted with adenovirus TG. Using group A Ad12 (Huie) genome as a probe no viral DNA was detected in 73 human tumors of which 33 were gastro-intestinal (GI). With virtually definitive EcoRI-C as probe no viral sequences were observed in the DNA of 85 tumors (including 37 GI) or in the RNA of 43 tumors (including 19 GI). Thus, Ad12 and probably all group A adenoviruses were not implicated in 99% of GI tumors. Using a group C adenovirus HindIII-G as probe, no viral sequences in the DNA or RNA of 116 tumors (including 61 lung and 38 colo-rectal) were detected. Thus group C adenoviruses definitely were not associated with 98% of lung or colo-rectal cancers. HindIII-G sequences were found in the DNA or RNA from 10 of 40 human tonsils. For the group B adenoviruses, Ad7 and Ad11 DNA probes were used to examine 80 tumors (including 36 lung and 19 GI) and tumors from 7 bladder and 4 prostate cancer cases. No viral DNA was detected, indicating that the group B adenoviruses are not major causes of lung cancer. Similar negative data was obtained when the DNA probe prepared from the group E adenovirus, Ad4, was used for 69 tumors (including 40 lung).

Using a very sensitive papovavirus (BK) probe, no viral DNA was detected in 150 tumors or 7 cultured human malignant cell lines.

Five major adenovirus early mRNA classes were isolated and mapped on four noncontiguous gene blocks, two on each DNA strand. The Ad2 TG block codes for 24S and 14S mRNAs in infected and transformed cells. 5RK cells express 50% of HindIII-G as mRNA; G-specific RNA is 32S in the nucleus but 14S in cytoplasm. HE C19 cells express about 80% of Ad12 genome as nuclear RNA, but 22% as mRNA, indicating posttranscriptional selection of viral mRNA. Ad2 early mRNA contains at least seven 5'-terminal methylated caps.

Eleven early proteins of Ad2 were identified using cycloheximide pretreatment and/or hypertonic treatment to enhance viral protein synthesis.

Ad2 75K single-stranded DNA binding protein (DBP) contains phosphoserine and exists in two size forms, 74K and 77K. Synthesis of DBP is inhibited at 11 hr post-infection. DBP is localized in the nucleus.

Significance to Biomedical Research and the Program of the Institute: This is a systematic study using the most sensitive techniques available to probe for evidence of an association between members of the adenovirus group and neoplasms occurring in humans.

Proposed Course: With the development of highly sensitive and specific DNA probes, major emphasis will be placed on the analysis of human tumors for the presence of adenovirus, papovavirus, and human papilloma virus specific nucleic acid sequences.

Date Contract Initiated: March 20, 1967

Current Annual Level: $500,000

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP6-1022)

Title: Studies of Human Wart Virus in Tissue Culture

Contractor's Project Director: Dr. Magdalena Eisinger

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To determine the antigenic properties of various human wart viruses (HWV) and the relationships of HWV to benign or malignant tumors.

Major Findings: Complement fixation, fluorescent microscopy and electron microscopy studies of Bean epithelial cells infected with HWV indicate that the ability of the virus to multiply in such cultures is dependent on the length of time such cells have been passaged in culture. Virus may only be recovered from cells infected at a low passage level.

An adaptation of Cikes' micro-complement fixation method for the studies of HWV antigens has proved to be a very sensitive and specific test for studies

of wart viral antigens produced in vitro. It was shown that in successfully infected cultures new virus antigen can be detected 4-5 days after infection, reaching a maximum on the 8-9th day.

CsCl density gradients of tissue culture materials revealed the presence of proteins identical with those of the purified HWV. The major viral capsid protein (m.w. 57,000) found in both wart and tissue culture preparations could not be detected in similarly treated uninfected control cells.

Successful replication of HWV appears to be dependent on cell maturation. An effort has been made to develop tissue culture systems in which cells will differentiate.

Epithelial monolayer cultures which continue to differentiate in vitro have been obtained with an adaptation of Freeman's organ culture technique.

When epidermodysplasia verruciformis virus was studied by complement fixation and immune-electron microscopy, evidence was obtained suggesting that the epidermodysplasia virus is antigenically distinct from both hand and plantar wart viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor's ability to propagate the human wart virus in quantities sufficient for in depth studies may prove to be the major factor in ultimately providing an opportunity to probe for definitive evidence for a link between the human wart virus and malignancy. In view of the prevalence of warts in the human population and the association of warts to some cancers in animals, the wart virus requires attention as a factor possibly contributing to mammalian neoplasia.

Proposed Course: This project will continue without change.

Date Contract Initiated: December 1, 1975

Current Annual Level: $116,000

SUMMARY REPORT

(5) RNA VIRUS STUDIES SECTION

July 1, 1976 through September 30, 1977

I. Viral Biology

New Nonhuman Primate Oncornaviruses: The search for new primate viruses continues to provide materials for production of new probes to search for evidence of type C oncornavirus information in human cells and to provide models for oncogenic virus epidemiology and control in species closely related to man. To date, the baboon endogenous virus discovered at Southwest Foundation for Research and Education has provided the best evidence for type C viral information in human cells. This contractor has reported preliminary characterization studies on another endogenous primate virus, the squirrel monkey retravirus (SMRV), a type D oncornavirus. The RNA dependent DNA polymerase of this virus has biochemical properties similar to that of Mason Pfizer monkey virus (M-PMV), and is antigenically related, but not identical, to the M-PMV polymerase. Nucleic acid hybridization and host range studies indicated that SMRV is an endogenous, xenotropic virus of squirrel monkeys. The pathogenicity of this virus has not been established.

Several new isolates of the gibbon ape leukemia virus (GaLV) were obtained at the University of California at Davis and epidemiological and pathogenesis studies were continued. Infected animals excreted infectious virus in urine and feces. Vertical transmission, however, has not been demonstrated. Plasma titers of infectious virus during the course of disease were shown to be inversely related to neutralizing antibody titers.

Search for Human Oncornaviruses: Biological, biochemical and immunological approaches were used to obtain evidence for oncornavirus information in human cells. Progress was reported in all areas in this year.

Virus-like particles were detected by electron microscopy in the trophoblast layer of 7 of 19 human placentas examined at the University of California at San Francisco. Furthermore, extracts of all 20 placentas examined were shown to contain RNA-dependent DNA polymerase. This activity sedimented in sucrose density gradients at 1.15 g/ml and at 1.24 g/ml. The activity was not neutralized by antisera to reverse transcriptases of known primate, feline, or murine type C viruses. Efforts to induce this virus in placental cultures are in progress.

Characterization of a reverse transcriptase enzyme purified from human breast cancer tissues by Spiegleman's group at Columbia University showed that the enzyme has a molecular weight of 70,000, prefers Mg^{++}, and effectively transcribes avian myeloblastosis virus RNA. The same enzyme is found in plasmas from patients with active disease. Radioimmunoassays for this enzyme are being developed to determine its antigenic relationship to known mammalian virus reverse transcriptases.

The complete purification of the reverse transcriptase isolated from particles in human leukemic peripheral white blood cells and spleens also was achieved by the group at Columbia University. The final product, which consisted of essentially one band in SDS-acrylamide gel electrophoresis, had a molecular weight of 70,000 daltons. A similar enzyme was purified from spleens of patients with chronic myelogenous leukemia. Radioimmunoassay for this enzyme is being developed to establish antigenic relationships to known mammalian viruses.

Proteins antigenically related to p30 and gp45 of the simian sarcoma virus (SSV-1) were not detected in malignant cells or body fluids of different leukemia cases examined by the Columbia group. On the other hand, evidence was obtained at the Sidney Farber Cancer Center and at Litton Bionetics, Inc. for the presence of nucleic acid sequences related to this virus in cells from patients with acute myelogenous leukemia. The previously described virus, HL-23, isolated from a case of acute myelogenous leukemia, was further characterized and found to be a mixture of SSV-1 and baboon endogenous virus (BaEV) (VCLA). Investigators at Litton Bionetics, Inc. also reported evidence obtained by immunological methods for virus-related information in cell strains established from normal human embryonic tissues.

Conditions were established allowing cultivation of human leukemic cells in vitro. Four cell strains were established representing an acute lymphocytic leukemia of abnormal T cells, an acute lymphocytic leukemia of "null" cells, an acute myelogenous leukemia of abnormal myeloid cells, and an acute myelogenous leukemia with B cell characteristics. Four new antigens of use in characterizing B, T, and leukemic cells were identified and antisera were prepared for use as probes. Cultures of these cells have yielded no evidence of the presence of an infectious virus.

A glycoprotein of molecular weight 55,000 was identified in membranes of human chronic granulocytic leukemia cells which showed evidence of relationship to a minor determinant on Friend Leukemia Virus gp71.

Interrelationships Between Cell Differentiation and Oncornavirus Infection:

Cancer has often been referred to as a disease of arrested cell differentiation. Increasing evidence of the role of oncornavirus infection in alterations of cell differentiation have made this area of research especially relevant to the goals of the Virus Cancer Program. Evidence obtained at the University of California at Berkeley in the chicken myeloblast-Rous Sarcoma Virus system showed that the effect of the virus on muscle cell differentiation was mediated through the control of cell-specific functions related to DNA synthesis and cell division, rather than through the process of fusion of myoblasts to myotubes.

Activation of Endogenous Viruses: Guinea pig retravirus was activated from strain 13 guinea pig leukemias by halogenated pyrimidine treatment (Microbiological Associates, Inc.).

A new amphotropic virus was isolated from clone A31 of Balb/c 3T3 mouse cells treated with IUdR. The host range of this virus was explored in detail at the Oak Ridge National Laboratory. The virus may be either the product of activation of an amphotropic virus or of recombination of simultaneously induced endogenous ecotropic and xenotropic viruses.

II. Antigenic Characterization Studies

Structural Aspects: Tryptic peptide maps of a number of gp70 MuLV glycoproteins isolated from virus purified from sera and excretions from a number of inbred mouse strains were constructed at the Scripps Foundation. Several classes of related virus envelope proteins could be identified. More than one type of gp70 could be isolated from a single mouse strain depending on the anatomical site of origin.

At the Albert Einstein College of Medicine, two new structural proteins, p14 and p17, were identified in Gross and Rauscher murine leukemia viruses. Investigators at the Fred Hutchinson Cancer Center identified a cell membrane polyprotein of molecular weight 85,000 daltons as the cell surface antigen reactive with antisera to MuLV p30. The usefulness of this reaction for identifying preleukemic cells is under investigation. MuLV gp71 was also identified as a cell surface antigen at Duke University. The importance of this antigen in immunoprophylactic studies is underway.

Precursor Protein Studies: Studies of precursor proteins were conducted by T. August and R. Arlinghaus to define the gene order in murine oncornaviruses. Analysis of precursors indicated that gag and pol genes are adjacent to each other. The order in gag was tentatively identified as NH_2 (p12, p30) p15 p10 - COOH. The major env protein precursor of Rauscher leukemia virus has a molecular weight of 90,000 daltons. A 60,000 molecular weight precursor to p30 was identified in all clones of ml-MSV transformed cells, but not in uninfected or leukemia virus-infected cells. Arlinghaus proposed a model for translational control and cleavage of virus precursor proteins.

III. Virus Expression During Cell Differentiation

The relationship between virus genetic expression and cell differentiation was studied at the Scripps Foundation and the Albert Einstein Medical Center. The gp70 antigen first appeared in hematopoetic liver of 14 day old embryos. Control of virus genetic expression in adult and developing mice possibly was linked to the differentiation of lymphoid and epithelial cells in some mouse strains. The gp70 and p30 proteins were not coordinately expressed. Different endogenous virus gp70's were expressed in different organs of the mouse. The level of gp70 in mouse serum varied among mice of different strains. The highest levels were detected in NZB mice; none was found in 129_{ix}- mice.

IV. Host response to viral antigens

The natural immune response of the host to endogenous virus antigens is determined by the presence of the virus antigen in host tissues (Scripps Foundation, Fred Hutchinson Cancer Center) as well as by host genetic factors

(New England Medical Center, Fred Hutchinson Cancer Center, Memorial Sloan-Kettering Cancer Center, Duke University).

Immunization of mice with purified gp71 demonstrated differences in the effectiveness of the response of mice of different strains, ranging from protection in low leukemia strains to enhancement of spontaneous disease in high leukemia strains. Passive immunization with anti-gp71 sera circumvented the undesirable reactions in high leukemia strains. Protection against challenge was afforded by the interspecies antigens of feline leukemia and Friend leukemia virus in vaccines and in passive immunization with antisera.

Two new inbred mouse strains were identified at the Scripps Foundation which develop a fatal lupus-like syndrome early in life.

Further study of immunoprotection of rats by a common tumor antigen recognized by antibody to a specific endogenous rat leukemia virus showed that significant prevention or suppression of polyoma virus transformation of rat cells could be achieved (Microbiological Associates, Inc.).

V. Molecular Virology

The definition of *in vitro* conditions allowing transcription of full-length DNA copies of oncornavirus genomes was achieved at Columbia University and MIT. At MIT, the *in vitro* DNA transcripts were shown to be biologically active by transfecting murine cells. Optimal conditions for transfection by nucleic acid were established at ERDA.

The mechanism of reverse transcription was studied by several contractors. tRNA requirements were shown to vary between the oncornaviruses derived from various species (ERDA). The β subunit was the component of reverse transcriptase active in tRNA binding (St. Louis University).

Progress was made in sequencing the genome of avian sarcoma virus from the 3' end (Massachusetts General Hospital). Evidence was attained for terminal redundancy between the 3' and 5' ends of virus RNA. This redundancy may be important in jumping from the 5' to the 3' end during transcription.

Sequencing at the 5' end of murine leukemia virus was begun at St. Louis University.

The structure of oncornavirus genomes was visualized by electron microscopy at the California Institute of Technology and shown to be similar for all mammalian, and possibly avian, oncornaviruses. Sequence homology between related murine sarcoma and leukemia viruses was studied. At the 5' end of the virus RNA, a homologous *gag* region was demonstrated. In the middle region a short non-homologous sequence was observed, possibly assignable to the *sarc* region. Circular heteroduplexes were often seen, and may provide a clue to the mechanism of reverse transcription.

Procedures for detection of specific virus sequences integrated into cell genomes were developed in the laboratories of Columbia University and the

California Institute of Technology. The structure of proviral sequences of simian sarcoma virus genes in their native conformation in the cell chromosome was shown to be organized in a nucleosomal structure analogous to host DNA.

The mechanism of host genetic restriction of Friend virus replication was pursued in two laboratories (ERDA, Albert Einstein College of Medicine). The data ruled out a block in translation or assembly, and transfection studies indicated that the restriction occurs within 6 hours of infection and involves interference with the synthesis of competent proviral DNA. The restriction may be mediated by an RNA.

In vitro conditions for translation of an 180,000 dalton polyprotein precursor were established at MIT. Transcriptional control in eukaryotic cells was studied in oncornavirus-infected mammalian cells. A field vole cell line transformed by avian sarcoma virus was found to exert post transcriptional regulation of viral structural and transforming genes (University of Minnesota). The effects of chemical inducers of erythroid differentiation on murine erythroleukemic cells transformed by Friend leukemia virus were also studied by Marks at Columbia University. The results indicated that several regulatory mechanisms are involved which respond differentially to several classes of inducers.

VI. Genetic Studies

Several contracts within the RNA Virus Studies Section have utilized a genetic approach towards studies of the possible etiologic role of endogenous type viruses in both spontaneous and chemically-induced tumors. One area of research in which this group of contracts has been involved is directed towards an analysis of the type C viral genome itself (USC). The approach taken has involved the isolation and characterization of temperature-sensitive and deletion mutants and the use of such mutants in efforts to construct a map of the type C viral genome. The results indicated that gag, a gene coding for several low molecular weight viral structural proteins, is located at the amino-terminal end of the genome. This is followed by a gene (pol) for the viral reverse transcriptase, a gene (env) for the envelope glycoprotein, gp70, and finally, at the carboxy-terminal end is a gene designated sarc, coding for transformation. Additional mutants are being developed for fine mapping studies.

Another major area of research in the Genetics group of contracts involves the identification and mapping of host genes influencing virus expression as well as determining host response to these viruses.

The emphasis in studies of the endogenous group of viruses has been on cellular regulatory genes influencing virus gene expression. One approach to this question involved efforts to develop series of strains of mice cogenic at loci which have been shown to be related to, or in some way influence, endogenous virus expression (Sloan-Kettering, New England Medical Center). Representative genetic loci being studied include H-2, PC, TL, and Fv-1, and Rvg-1. The second approach involved the identification of loci influencing xenotropic virus expression by genetic crosses between inbred mouse strains characterized

by differing levels of endogenous xenotropic virus expression (Jackson Labs.). Carcinogen induction of tumors was shown to also be genetically determined, and related to the loci governing viral expression. In the avian system, several cellular genes influencing expression of the endogenous avian type C virus, RAV-O were identified (USDA). Moreover, using this system, preliminary genetic evidence indicated that elevated levels of expression of virus may be associated with increased susceptibility to spontaneous tumor development.

If, as suggested by these preliminary findings, endogenous type C viral expression is found to be an important determinant of malignant transformation, it will be of obvious importance to determine means of suppressing or interfering with this process. In an examination of systematic factors influencing virus expression, the immunologic responses of the host to endogenous viral gene expression was studied (Jackson Labs., New England Medical Center). Identification of two new immune response genes specific for type C viral antigens was accomplished.

RNA VIRUS STUDIES SECTION

Dr. Wilna A. Woods, Head

AGRICULTURE, DEPARTMENT OF (YO1-CP4-0214)

Title: Inheritance and Oncogenicity of RNA Tumor Viruses

Contractor's Project Director: Dr. L. B. Crittendon

Project Officer (NCI): Dr. John Stephenson

Objectives: These studies make use of inbred chicken lines to define the genes involved in controlling expression of the infectious endogenous type C RNA chicken viruses (RAV-0) and will determine whether the RAV-0 virus is oncogenic.

Major Findings: Progress was made in the development of assay procedure for endogenous virus expression, the characterization of inbred chicken lines for virus expression, and the characterization of lymphoid tumors which have occurred in one SPF line.

Sensitive assays for RAV-0 were developed based on a mixed tissue culture of non-producer RSV-quail cells (23-16) and turkey cells; the supernatant fluids from these cultures were assayed on cells susceptible to virus of subgroup E. Chick-helper-factor (chf) was detected by fusing the test cells with 23-16 cells, using killed Sendai virus. Twelve chicken lines and sublines were screened for spontaneous RAV-0 production. RAV-0 was produced in two inbred lines which had not been previously studied. Therefore, a total of four independent inbred lines produced RAV-0 at high levels; RAV-0 has seldom been detected in outbred stock.

Several cases of lymphoid leukosis occurred in RPRL line 7 over the past year. These birds appeared to be free of exogenous leukosis virus expression, but produced RAV-0 spontaneously. Subgroup E virus was grown from tumor material derived from two birds. No other subgroup of virus has been detected. Exogenous proviral DNA in the tumor material was not demonstrable; in contrast, proviral DNA was found in tumors of birds infected with exogenous leukosis viruses. The role of this RAV-0 in the genesis of these tumors was being investigated by tests in vivo and in vitro for transformation and infectivity.

Significance to Biomedical Research and the Program of the Institute: It is increasingly apparent that many vertebrates, including primates, carry information for complete RNA tumor virus expression, including oncogenic potential, in their genomes. The endogenous origin of this viral information changes our concepts of the control of at least some types of cancer from methods which eliminate or control exogenous viruses to mechanisms which can suppress endogenous viral information. These studies provide another experimental system, in addition to the mouse, where the genes controlling production of the endogenous type C virus will be defined. They will provide another system for defining the role of the endogenous virus in naturally occurring neoplasms.

Proposed Course: Continue to study the influence of endogenous and exogenous RAV-0 and the presence of natural gs antigen on the incidence of lymphoid leukosis in adult chickens.

Date Contract Initiated: July 1, 1974

Current Annual Level: $29,000

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP3-3311)

Title: Studies on the Molecular Basis of Viral Carcinogenesis

Contractor's Project Director: Dr. J. Thomas August

Project Officer (NCI): Dr. John Stephenson

Objectives: To isolate and purify structural viral proteins and virus-associated enzymes; characterize their biological, chemical, and immunological properties, and use them as probes for analysis of viral gene expression, in tumor tissues, as agents for possible immunological control of viral infection, and as reagents for detection of virus infection.

Major Findings:

A. Purification and Characterization of Viral Proteins

Purification and Characterization of Gross Murine Leukemia Virus Proteins: The Gross virus glycoprotein of 71,000 daltons and proteins of about 30,000. 17,000, 14,000, 12,000 and 10,000 daltons have been purified to homogeneity and used to prepare immunospecific antisera in goats.

Antigenic determinants of p12 were chiefly subgroup-specific with a close homology to the analogous protein of AKR, Kirsten, or BALB/c xenotropic or NZB xenotropic type C viruses. Weak interspecies cross-reactivity was demonstrated with the Theilen feline virus, but very little, if any, with different primate oncornaviruses.

Structure and Chemistry of the Viral Envelope Glycoprotein: The 69,000 and 71,000 dalton glycopeptides of Rauscher murine leukemia virus were shown to be nearly identical by tryptic mapping, immunochemical and virus neutralization analysis. In addition, other minor virion glycoproteins of 47,000 and 35,000 daltons are found to be degradation products of the major glycoprotein.

Anti-Rauscher virus gp69/71 serum also precipitated other lower molecular weight polypeptides. A small polypeptide of about 15,000 to 17,000 daltons is receiving particular attention.

In Vitro Labeling of Virion Glycoproteins: External labeling of sialic acid or galactose residues of Rauscher virus showed that in every case the major component labeled was a glycoprotein of about 70,000 daltons.

Genetic Expression of Virus Reverse Transcriptase: Rauscher virus reverse transcriptase has been purified to homogeneity and a radioimmunoassay for analysis of the expression of this protein in cells and tissues is being developed.

Virus Precursor Proteins: Anti-Rauscher virus p30 serum and anti-p15 serum precipitated a pulse-labeled protein of 65,000 daltons (Pr65) as the major virus-specific polypeptide. In addition, polypeptides of 350,000, 260,000, 76,000 and 60,000 daltons were precipitated by both antisera. With chase, a decrease in the concentration of the labeled polypeptides of large and intermediate sized was observed and p30 protein appeared. Tryptic digest analysis showed that the 65,000 dalton precursor contained the peptides of p30 and p15.

Anti-Rauscher virus gp69/71 serum precipitated a pulse-labeled protein of 90,000 daltons (Pr90) as the major envelope glycoprotein-specific polypeptide. In addition, this serum precipitated the same 350,000 dalton and 260,000 dalton polypeptides as did the anti-p30 and anti-p15 sera. Labeling experiments using tritiated sugars showed that Pr90 was a glycoprotein, and tryptic digest analysis of Pr90 confirmed its role as precursor to gp69/71.

Virion Protein Kinase: The virion protein kinase of Fv-1 has been purified to homogeneity and shown to be virus coded by chemical, enzymatic, immunochemical and genetic criteria.

B. Control of Viral Gene Expression

Induction of Viral Gene Expression in Somatic Cell Hybrids: Cell hybrids of BALB/c and C57BL/6 mouse peritoneal macrophages with human Lesch-Nyhan fibroblasts transformed by infection with simian virus 40 (SV40) were examined for synthesis of type C virus proteins.

Hybrid clones of BALB/c cells containing human chromosome 7 and the SV40 genome were induced to synthesize extremely high concentrations of murine virus core and envelope proteins.

Oncornavirus Gene Expression During Embryonal Development of the Mouse: BALB/c mouse tissues contained very low concentrations of both p30 and gp70 in early embryos and adult tissues, whereas moderate levels of both were present in late embryos and newborn animals. C3H mouse tissues contained high concentrations of glycoprotein throughout embryogenesis and in the newborn and adult spleen; low levels of internal protein were also present throughout. AKR mice contained minimal amounts of glycoprotein in embryos, increased concentrations in the newborn, and high levels in the adult spleen; the major internal protein was present in low levels throughout embryogenesis and in the newborn and rose to high levels in the adult spleen. With these studies, no pattern of viral protein expression that can be attributed to a developmental process was detected.

C. Sarcoma Virus Gene (Sarc) Expression

An extensive analysis has been carried out of cloned cell lines of Kirsten sarcoma virus (KSV)-transformed normal rat kidney (NRK) cells that also express

the viral glycoprotein, but no other viral protein. KiSV failed to induce morphological transformation in NRK cells previously infected with either Rauscher or Kirsten murine leukemia viruses. In contrast, viruses which do not belong to the murine ecotropic group, the endogenous rat virus, or the primate woolly monkey virus (SSV-1) did not interfere with superinfection and focus formation by KSV.

Cell clones positive for virus envelope antigen were resistant to superinfection with either of the two strains of murine ecotropic viruses tested, whereas envelope antigen-negative cells were readily infected. These experiments clearly demonstrated that interference to infection was mediated by the virion envelope glycoprotein.

A series of experiments designed to isolate $sarc^-$, $p30^-$, env^+ cells by nonselective cloning of normal cells from focus assay plates yielded negative results as did attempts to increase the frequency of isolation of env^+ cells by infection at high multiplicities; this argued against a dual infection with a defective leukemia virus.

The model best supported by the present data is that $sarc^+$ cells were infected with a virus which contained both the env and sarc genes. The chief evidence for this was that analysis of the phenotype of cells, transformed by sarcoma virus rescued from env^+ transformed cells, showed that the majority, but not all, of the transformed clones expressed the murine viral envelope antigens.

D. RNA Tumor Virus Genetic Expression

Oncornavirus Envelope Glycoproteinemia: Virus envelope glycoprotein detected by radioimmunoassay was found in every mouse strain except the $129G_{IX}$-strain.

Competitive displacement in assay of gp69/71 could readily be detected with serum of almost all strains of mice tested. Among the mice tested, using unpurified serum, the antigen in BALB/c strains was at the lower limit of detection. Moreover, from the extent of competition, it appeared that the competing components contained most, if not all, of the interspecies antigenic determinants of the Rauscher virus gp69/71. Serum p30 protein was found in high leukemia mouse strains and in New Zealand mice, but not in any of the low leukemia mouse strains. Moreover, the concentration of p30 in the high incidence strains, and particularly in the NZB mice, was much lower than the concentration of envelope glycoprotein. The results, therefore, suggested that, in general, and particularly with NZB and low leukemia mice, the serum concentration of envelope glycoprotein is subject to some special synthetic or metabolic process, probably not directly related to synthesis of virions.

Natural antibodies to purified Rauscher leukemia virus core or envelope glycoproteins or to Gross virus glycoprotein were not detected in serum from any mouse strain examined.

E. Studies on the Synthesis of Oncornavirus RNA and on RNA Processing in Eukaryotic Systems

Transcription of Viral RNA: Products formed by exogenous RNA polymerase during transcription of chromatin isolated from myeloblasts of chickens infected with avian myeloblastosis virus contained viral-specific sequences. The characteristics of the in vitro transcription system were examined. The results are consistent with the suggestion that localized unwinding of DNA is required for gene expression.

Double Strand Specific RNase: A eukaryotic RNase specific for double-stranded RNA has been isolated and characterized. An activity purified approximately 1300-fold from calf thymus cleaves double-stranded RNA nearly 20 times more effectively than single-stranded RNA.

Capping of mRNA. (a) GTP:mRNA Guanylyl transferase has been isolated from vaccinia virus and purified at least 70,000-fold. The characteristics of the enzyme and the reaction were examined. (b) GTP:mRNA Guanylyl transferase was solubilized from nuclei isolated from calf thymus.

Significance to Biomedical Research and the Program of the Institute: Specific protein products of virus genetic expression, purified and well characterized, provide the reagents to examine relationships between viruses in terms of gene products and to search for evidence of similar viral gene functions in human disease.

Secondly, these sub-virion proteins provide the means to determine whether effective immunological control of virally-induced disease could be instituted without complication by viral genetic material.

Proposed Course: This contract terminated April 30, 1977.

Date Contract Initiated: April 26, 1971

Current Annual Level: $203,000

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP4-3380)

Title: Host Restriction of Friend Leukemia Virus

Contractor's Project Director: Dr. Ruy Soeiro

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To elucidate the molecular mechanism of host restriction of Friend leukemia virus. Specific steps in viral replication will be analyzed to determine those which are affected by the Fv-1 gene restriction. Studies focus on (1) restriction of transcription of viral RNA, (2) failure of stable integration of viral DNA and (3) inhibition of translation of viral proteins.

Major Findings: Virus protein synthesis was investigated in the nonpermissive host cell by immunoprecipitation and by the formation of pseudotypes of vesicular stomatitis virus (VSV). The latter approach was directed to virus envelope glycoprotein; the former to total virus proteins. The conclusion reached by both methods was that virus protein synthesis is dramatically reduced in the nonpermissive cell.

Virus RNA synthesis was followed in the nonpermissive cell infection. The results indicated that total, nuclear, and polysomal messenger RNA were all decreased in amount by comparison with the permissive cell infection.

These data appeared to rule out a block in either virion assembly or translation as the mechanism for Fv-1 restriction.

Lastly, the fate of input genome RNA was re-investigated. The results from a series of biological studies again suggested that some portion of the input virus genome RNA gene integrates with host cell DNA.

Significance to Biomedical Research and the Program of the Institute: Genetic susceptibility to cancer in humans is demonstrated by many examples. A partial listing of apparently recessive conditions includes those associated with visible chromosomal disorders. Downs syndrome children have a 10-20 fold increase in incidence of leukemia. Other trisomic states (trisomy D and Kleinfelter's syndrome) also have an increased incidence of leukemia. Xeroderma pigmentosum, and autosomal recessive inherited syndrome, is associated with a high incidence of skin cancer. Fanconi's syndrome, an autosomal recessive trait, increases risk of leukemia in both homozygotes and heterozygotes. Familial cancers with apparent dominant gene(s) resulting in a highly penetrant genetic predisposition to cancer include the neurofibromatoses, retinoblastoma and other embryonal tumors, pheochromocytoma, and polyposis of the colon. Common cancers including breast, uterine, gastric and lung cancers all appear in increased incidence in some families.

The availability of the murine oncornavirus system, with congenic lines of mice differing in specific loci known to be associated with resistance or permissiveness to leukemia virus replication, serves as an excellent model to study genetic control of tumor virus replication.

Proposed Course: Studies on the fate of input viral RNA and on the synthesis of new viral DNA and proteins will be extended with emphasis on the comparison of the replication of the Friend leukemia virus in restrictive versus permissive host cells.

Date Contract Initiated: June 25, 1974

Current Annual Level: $79,015

CALIFORNIA INSTITUTE OF TECHNOLOGY (NO1-CP4-3306)

Title: Electron Microscope Studies of Tumor Virus Nucleic Acids

Contractor's Project Director: Dr. Norman Davidson

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To define the structure of oncornavirus RNA and the position of the integrated viral genome in cellular DNA by biochemical, biophysical and electron microscopic techniques.

Major Findings: Observation of a dimer structure, previously observed in RD-114, BKD and woolly monkey virus RNAs, was observed also in RNA from both ecotropic and xenotropic murine viruses. These observations strongly support the hypothesis that this is the general structure of the genome of all mammalian type C RNA tumor viruses and presumably of the avian type C viruses as well. There was a secondary loop structure with an inverted repeat as its stem approximately in the middle of all of the monomer units. There may be detailed hairpin features and crossovers within the loop that are characteristic of one genome and not of another. One of the principal differences between one dimer and another was the ease of dissociation into monomers. The RD-114 and BKD viruses were much more stable than all the other viruses which had roughly similar stabilities. Several sarcoma virus-leukemia virus mixtures were studied; in each case, the sarcoma virus was recognizably smaller. In none of the cases studied, however, was there a significant number of heterodimers.

A new program was initiated on the study of the sequence relations betwen related but genetically different RNA tumor viruses. As a part of this research, the sequence relations between one isolate of Moloney-MSV and the parental Moloney-MLV were studied. Beginning at the 5' end, the two RNA's were homologous for a region 2.25 kb. in length. This region may be assigned to the gag gene (internal structure proteins) which are known to be present in MSV as well as MLV. In the middle region of the genome, there were several short nonhomologous features as well as some duplex sequences. A moderately long sequence (2.9 kb) close to the 3' end of the MLV RNA was replaced by a slightly shorter region (1.5 kb) in MSV. The 1.5 kb MSV segment presumably contained the sarcoma gene. The total region probably contained the envelope gene and the reverse transcriptase gene. It seemed highly probable, however, that the sarcoma gene present on MSV and not in MLV was in the β_S sequence. At the 3' end, there was an additional duplex region, α, which, perhaps, is analogous to the "common sequence" present in avian viruses. The fact that circular heteroduplexes were often seen showed that cDNA transcription was initiated somewhere on a primer in the interior of the molecule (actually, fairly close to the 5' end according to other experimental data), then somehow jumped to the 3' end and proceeded around back to the primer.

In preparation for the isolation of integrated virus DNA sequences, conditions of hybridization were discovered under which the rate of RNA:DNA hybrid formation was much greater than the rate of DNA:DNA duplex formation. This was achieved by working in solvents containing a very high percentage of formamide at conditions close to the T_m of the DNA. In addition, further studies were

made on the chemical modification of RNA so as to attach an affinity chromatography label for use in gene enrichment experiments for integrated viral DNA sequences.

Significance to Biomedical Research and the Program of the Institute: Successful application of the proposed methodology will make it possible to determine precisely where the cancer virus genome is integrated into the transformed cell DNA; i.e., whether present at one site or at multiple sites; whether different defined portions of the genome are in different portions of the cell. The approach used permits direct visualization of the degree of homology between viral RNAs and is particularly important for the comparative study of endogenous viral information and exogenous virus infection in relation to the transformation process and tumor development.

Proposed Course: The contractor will (1) study, by electron microscopy and other physical methods, the structure of the high molecular weight RNA extracted from RNA tumor viruses, particularly to determine the generality of the dimer structure observed for RD-114 and several other viral RNA's. The nature of the bond between the two monomers will be determined to find out if there is a cross-linker molecule (either a protein or a small RNA). (2) Develop a heteroduplex method, using a cDNA transcript from one viral RNA, to map the regions of homology and of nonhomology between the RNA molecules of related tumor viruses. The sequence relations between different related tumor virus RNA's will be systematically studied.

Date Contract Initiated: March 1, 1974

Current Annual Level: $110,315

CALIFORNIA, UNIVERSITY OF (NO1-CP3-3283)

Title: Studies on the Putative Role of RNA Tumor Viruses in Human Tumor Cells

Contractor's Project Director: Dr. Marcel Baluda

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To characterize virus-related DNA sequences detected in human cell DNA and determine whether they are transcribed in human tumors; characterize new isolates of putative human oncornaviruses; determine whether these can be used to probe for the detection of putative human oncornavirus-specific DNA sequences; and investigate whether the oncornavirus DNA sequences are restricted to neoplastic cells or uniformly distributed in all tissues in cancer patients and whether transcription of virus-specific DNA sequences is limited to the tumor cells.

Major Findings: The simian sarcoma-associated virus (SSAV) genome subunit is slightly smaller than that of avian myeloblastosis virus (AMV) and virions contain a high proportion of cellular ribosomal RNA. These findings may explain why earlier studies using 60S RNA from purified SSAV virions yielded a relatively high degree of hybridization (20-50%) of the viral RNA with DNA from normal or

malignant tissues. The use of 28-30S subunits melted from 60S RNA isolated from purified SSAV gives a much lower percentage of hybridization of viral RNA with DNA from normal or neoplastic human tissues.

The HL-23 virus, isolated by Gallo from a patient with acute myelogenous leukemia, was studied. The size of the RNA subunit appeared to be the same as that of SSAV (28-30S); aggregates of 50-60S appear if the viral RNA is analyzed prior to melting DNA sequences complementary to both SSAV and HL-23 virus RNA were detected in cells from normal individuals and cancer patients. Preliminary results indicated that the complementary DNA sequences may be present in some cells but that these sequences represent either a small fraction of the virus genome or are present in only a small fraction of the cells in the tissues tested.

The RNA of the HL-23 virus was found to represent a mixture of SSAV and Baboon endogenous virus (BaEV) but not a recombinant between these two primate viruses. DNA from either normal or malignant human tissues, including 17 leukemias that were tested, appeared to contain the same level of base sequence homology with 30S RNA from BaEV. Also, there was no detectable difference between normal and malignant human DNA when I^{125}-labeled 70S BaEV RNA was used as the hybridization probe. However, with the latter probe, approximately twice as much viral RNA appeared to hybridize as with ^{3}H-labeled 30S BaEV RNA.

Significance to Biomedical Research and the Program of the Institute: The ability of RNA tumor viruses to induce neoplasia in different species of animals with high efficiency is well established and recent evidence suggests that similar viruses may be involved in human cancer. The presence in human cancer cells of type B and C particles, of nucleotide sequences with homology to viral RNA, and of protein antigens related to oncornavirus subviral components has been reported from different laboratories. This contract explored these relationships to determine the nature of the virus-like components in human cancer cells.

Proposed Course: This contract terminated May 31, 1977.

Date Contract Initiated: June 8, 1972

Current Annual Level: $174,871

CALIFORNIA, UNIVERSITY OF, BERKELEY (NO1-CP5-3537)

Title: Transformation of Differentiating Cells

Contractor's Project Director: Dr. G. S. Martin

Project Officer (NCI): Dr. Charles Sherr

Objectives: To investigate the relationship between transformation by RNA tumor viruses and the differentiation of the target cell.

Major Findings: The effects on myogenic differentiation of transformation by a temperature-sensitive mutant of Rous sarcoma virus, tsLA29, were studied. When chick muscle cells infected by this mutant were shifted from 35° to 41°, they reverted to a phenotypically normal state and fused into myotubes. If the cells were shifted from 35° to 41° under conditions where fusion is blocked by low-calcium medium, the cell differentiated into elongated myoblasts, similar to those found in uninfected cultures grown under the same conditions.

The synthesis of myosin was measured in cultures transformd by tsLA29. Both under normal (fusing) conditions, and in conditions where fusion was blocked, the synthesis of myosin was markedly stimulated by a shift from 35° to 41°. Cells infected by the wild type virus, Prague RSV, synthesized very little myosin at either temperature. The myosin, synthesized at 41° in cultures infected by tsLA29, was identified as skeletal muscle myosin by immunoprecipitation, using an antibody specific to the skeletal muscle form of myosin.

The synthesis of DNA in infected myoblasts was studied using ^{3}H-thymidine labeling and autoradiography. Synthesis of DNA in myoblasts infected by tsLA29 ceased within 24 hours after a shift from 35° to 41°, both in complete and in low-calcium medium. The cessation of DNA synthesis in the myoblasts appeared to be a specific response: it was not due to medium depletion, since the fibroblasts in the infected cultures continued to grow after DNA synthesis had ceased in the myoblast population.

These observations indicated that the virus exerted effects on muscle cell differentiation which were not due solely to an effect on the process of fusion. They were consistent with the hypothesis that the effects of the virus on cell-specific functions are due to maintenance of DNA synthesis and cell division.

Significance to Biomedical Research and the Program of the Institute: Many people refer to cancer as a disease of cell differentiation. The degree to which a tumor cell retains histological evidence of differentiation is often used by pathologists as an indication of its state of malignancy. Furthermore, if tumor cells retain some of the growth characteristics, such as hormone dependence, of the parent cell, these properties can be of great importance in therapy. Often, if malignant cells undergo terminal differentiation, their malignant characteristics are lost. Thus, this study of viral transformation of differentiating cells represents an approach which may be relevant to both diagnosis and therapy of cancer.

Proposed Course: The objective of this research is to study the relationship between transformation by RNA tumor viruses and the differentiation of the target cell. These studies will use as a model system a line of myogenic cells derived from embryonic rat muscle. These cells will be infected with avian and murine sarcoma or leukemia viruses, and by viral mutants which are temperature-sensitive in their ability to transform. The effects of transformation on subsequent differentiation and the effects of transformation on a cell which has already undergone terminal differentiation will be studied using the synthesis of "muscle-specific" proteins to monitor the state of differentiation of the cell. Specifically, the synthesis and activity of acetylcholinesterase will be investigated to determine if transformation can affect a differentiating trait that is already being expressed.

Date Contract Initiated: July 1, 1975

Current Annual Level: $53,610

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CP3-3242)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Thomas G. Kawakami

Project Officer (NCI): Dr. Wade P. Parks

Objectives: To detect, isolate and characterize type C viruses from spontaneous malignancies of primates and humans.

Major Findings: All human and primate tissue specimens examined, except those from gibbons, yielded no evidence of an infectious type C virus. Three healthy zoo gibbons were found to be infected with type C virus. Partial characterization of the new isolates indicated that the morphological, biophysical and antigenic properties were closely related but not identical to the previously described gibbon/woolly monkey type C virus complex. DNA copies complementary to the gibbon virus RNA were prepared by the endogenous reverse transcriptase reaction and found effective for differentiating between normal and virus-infected cells and tissue.

Molecular hybridization techniques were used to compare the nucleic acid relationships between the various primate type C viruses isolated from diseased and normal gibbons. Comparison of the genomes of a virus newly isolated from a normal gibbon with previously isolated virus revealed both closely related and unrelated nucleotide sequences. The proviral information could be readily detected in virus-infected tissue culture cells and in neoplastic tissue of viremic animals but not in nonneoplastic tissue.

Gibbons from other laboratories and zoos were found to have been exposed to the gibbon type C virus. Viremic gibbons were found to excrete infectious virus in urine and occasionally in feces.

Gibbons (20) infected with type C virus were monitored for viremia, immune response, clinical changes and alteration in the hematopoeitic cells. Viremic gibbons showed an increase in leukocyte counts with the major increase in the granulocytes. These animals lacked detectable humoral antibody reactive with the virus antigens although immune globulins were detected by electrophoresis and immunodiffusion tests. Electrophoretic analyses of serum proteins from normal, antibody positive, viremic and leukemic gibbons indicated that leukemic gibbons developed an increase in the immunoglobulins. Sera from gibbons with natural antibody could neutralize the viral reverse transcriptase. The virus in sera from viremic gibbons could infect T-lymphocytes as well as non-T-lymphocytes. A young gibbon inoculated with the gibbon leukemia virus developed an immune response to the virus infection. Following appearance of neutralizing antibodies, the antibody titer decreased with a resultant recurrence of persistent plasma viremia. Lymphocyte stimulation did not occur

during the secondary viremic state. The second hematological change which involved the appearance of increased numbers of immature and mature granulocytes coincided with the enlargement of the spleen and liver, and periosteal proliferation. Periosteal proliferation was confirmed by radiographs. Based on the hematologic and clinical changes in the experimental animal, which were similar to previously diagnosed granulocytic leukemia (HLA-25), the animal was diagnosed to be preleukemic or leukemic.

New gibbon sera obtained from a pet and a zoo animal were found to have antiviral antibody while other apes and humans lacked evidence of humoral antibodies specific for this primate type C virus.

Virus transmission studies indicate that the gibbon virus is not vertically transmitted to the offspring of antibody positive or noninfected mothers. Two offspring of a plasma viremic mother in a zoo were found to be viremic. Only plasma viremic gibbons were found to excrete infectious virus in urine, but no virus was detected in fecal or saliva samples from the same animals. Mosquitoes (Aedes aegypti) fed on high titer plasma viremic gibbons did not harbor, nor replicate, infectious virus after the blood meal.

Significance to Biomedical Research and the Program of the Institute: This gibbon colony offers a unique opportunity for the study of type C virus-associated leukemias spontaneously occurring in an ape. The virus materials obtained are important as probes for related components in human cancer cells. Comparative study of normal animals with those that develop disease provides opportunity to obtain some insight into host factors related to expression of disease.

Proposed Course: (1) Continue efforts to detect and isolate oncogenic agents from spontaneous tumors of primates and humans utilizing sensitive in vitro techniques. (2) Characterize newly isolated primate viruses and virus-like particles in human tumors and placentas. (3) Expand epidemiologic studies on primate viruses to determine animal carriers of the primate viruses. (4) Continue pathogenesis studies with primate viruses in primate species with special emphasis on the gibbon viruses. (5) Continue studies on the immune response (humoral and cell-mediated) in gibbons.

Date Contract Initiate: November 1, 1969

Current Annual Level: $431,125

CALIFORNIA, UNIVERSITY OF, SAN FRANCISCO (NO1-CP4-3381)

Title: Isolation of Human Xenotropic Viruses

Contractor's Project Director: Dr. Jay A. Levy

Project Officer (NCI): Dr. Takis Papas

Objectives: (1) To establish placental cell lines from patients with systemic lupus erythematosus (SLE) and lymphocyte cell lines from patients with acute leukemia. Cocultivate the human cells with cells from different animal species to attempt virus recovery. (2) To assay sera from patients with SLE and leukemia for antiviral reactivity.

Major Findings: Nine cell cultures from 5 human placentas were established. Evidence of reverse transcriptase-containing virus presence in supernatant culture fluids could not be demonstrated by biochemical, biological, or electron microscopic assay.

Virus-like particles were detected by electron microscopy in the trophoblast layer of 7 out of 19 placentas. No difference in incidence of these particles was observed between normal placentas and those from patients with SLE.

Fractions of extracts of 20/20 placentas obtained by density gradient centrifugation were shown to contain RNA-directed-DNA polymerase (reverse transcriptase) activity at densities of both 1.15 g/ml and 1.24 g/ml. This activity was demonstrated with poly rc oligo·dG_{12-18} and poly (rCm)oligo·dG_{12-18} synthetic templates. The reverse transcriptase (RT) activity at a density of 1.15 g/ml changes to a density of 1.24 g/ml after heating. These observations suggested conversion of enveloped viruses to viral cores. The RT activity was not neutralized by antisera to the RT of known primate, cat, and mouse type C viruses. The human placental enzyme has a cation preference for MG^{++}. These data suggest that the human placental virus is similar to a type D virus.

Significance to Biomedical Research and the Program of the Institute: Antigens cross-reacting with antisera specific for the p30 polypeptides of Rauscher murine leukemia virus have been reported to be present in specimens from cases of lupus erythematosus, and virus resembling type C particles has been observed budding from human and monkey placental tissue. This project is designed to determine whether a virus can be isolated from such human tissues by methods successfully applied in animal systems. A virus isolated from SLE patients would be extremely valuable for studies on autoimmune disease and leukemia in humans in analogy with the condition observed in NZB mice.

Proposed Course: The contractor will continue studies on the virus-like activities in placentas of women with multiple pregnancies and women with SLE and examine these placentas and cell lines for virus-like particles by particles by electron microscopy. The reverse transcriptase (RT) activity in placentas from multiparous and primiparous women will be compared. The RT activity in human placental material will be characterized to determine its cation preference and specific activity.

Studies on the endogenous polymerase activity of the placental extracts will continue. DNA synthesized by the RTs found at different densities will be compared. Hybridization of this DNA with DNA and RNA from human cells and/or from cells of primates or other animals will be determined.

An antibody to purified placental RT will be produced. With this reagent, the antigenicity of the placental RT will be compared to other DNA polymerases from normal human cells and other reverse transcriptase-containing viruses. Attempts

to cultivate virus from extracts and/or cell cultures of human placentas will continue.

Biologic and biochemical studies on extracts of placentas from patients with SLE will continue. Comparison of the RT activity in SLE placentas with that in normal placentas will be concluded.

Date Contract Initiated: June 27, 1974

Current Annual Level: $122,836

COLUMBIA UNIVERSITY (NO1-CP6-1008)

Title: Transcriptional Regulation of Eukaryotic Gene Sequences

Contractor's Project Director: Dr. Paul A. Marks

Project Officer (NCI): Dr. Wade Parks

Objectives: The overall objectives of this contract are to investigate the regulation of gene transcription in oncogenic virus-infected murine cells which have the potential for erythroid differentiation, specifically, to define factors which affect the transcription of structural genes characteristic of the differentiated state of Friend virus-infected murine erythroleukemia cells, namely, α and β globin genes. The expression of differentiated transcription programs in both fetal mouse erythroid cells as well as in the Friend virus-infected murine erythroleukemia cells will be characterized.

Major Findings: During the initial six months of this contract, attempts were made to define the chemical characteristics of compounds which are effective as inducing agents that cause mouse erythroleukemia cells (MELC) to undergo erythroid differentiation. Analysis of alterations in the cell cycle during differentiation of murine erythroleukemia cells induced by dimethylsulfoxide and other agents was undertaken. The rate of DNA synthesis, proportion of cells in S-phase and the pattern of DNA accumulation were measured and compared in MELC grown in the presence and absence of dimethylsulfoxide and other inducing agents (e.g., butyric acid and dimethylacetamide). There was evidence that dimethylsulfoxide (ME_2SO) and other inducing agents transiently inhibited the entry of cells into the S-phase of the cell cycle. This inhibition of progression through the cell cycle was detected in non-synchronous cultures as early as 5 hours and was maximal about 20 hours after beginning of culture.

In experiments currently in progress, evidence was obtained which suggested that there are configurational and/or structural changes in DNA from cells of MELC cultured with ME_2SO for a period of at least 25 to 30 hours. These changes in DNA are characterized by a decrease in the rate of sedimentation in alkaline sucrose gradient. No change was observed in the rate of DNA sedimentation in neutral sucrose gradient. Further, analysis of the DNA recovered from fractions from the alkaline sucrose gradient suggested that there may be an accumulation of single strand nicks in DNA from Me_2SO-induced cells.

The effects of inducers such as ME_2SO, hexamethylene bisacetamide (HMBA) as well as butyric and propionic acids on expression in MELC of the two beta globin genes. beta-major (βmaj) and beta-minor (βmin), during the course of differentiation have been examined. After four days of culture with HMBA or ME_2SO, the βmaj containing hemoglobin, called hemoglobin major (Hbmaj), predominated. By contrast, in the presence of butyric or propionic acid, after four days of culture, roughly equal amounts of Hbmaj and Hbmin were found. When poly (A)-containing RNA (mRNA) from MELV exposed to different inducers was translated in a cell-free wheat germ protein-synthesizing system, the ratio of βmaj to βmin globin synthesized reflected the ratio of globins synthesized in the intact cells. In MELC strain DR-10, resistant to induction by Me_2SO, the preferential stimulation of βmaj synthesis by HMBA and of βmin synthesis by butyric acid was even more pronounced. These studies suggested that there are regulatory mechanisms which respond differentially to the several classes of inducing agents, resulting in differential rates of expression of the structural genes involved in erythroid cell differentiation.

Significance to Biomedical Research and the Program of the Institute: This contract should provide better understanding of the control of transcription in oncogenic virus-transformed cells. More specifically, these investigations should lead to the identification of chemicals that can, in a predictable manner, alter the expression of genes in virus-transformed erythroleukemia cells and provide an understanding of mechanisms involved in inducing virus-transformed cells to differentiate and lose oncogenic properties.

Proposed Course: The contractor will investigate, specifically, (1) the chemical structure of compounds effective as inducing agents with particular emphasis on elucidation of the nature of the chemical structure required to effect the transcription of genes in these virus-transformed cells; (2) alterations in chromatin and DNA associated with induction of virus-infected cells to express erythropoietic differentiation; (3) studies of the action of inducing agents on the rate of accumulation of products of transcription of specific structural genes (e.g., α' βmaj and βmin globin genes); and (4) study of the relative complexity of the transcriptional products in virus-infected mouse erythroleukemia cells during induction of differentiation variants.

Date Contract Initiated: June 27, 1976

Current Annual Level: $162,782

COLUMBIA UNIVERSITY (NO1-CP6-1010)

Title: Replication of Oncogenic RNA Viruses and its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Takis Papas

Objectives: To explore the possible involvement of RNA tumor viruses in human neoplasia and to exploit any leads that emerge for use in the diagnosis and therapy of cancer.

Major Findings:

The Mechanism of Action in Reverse Transcriptase: Conditions were developed permitting synthesis of complete, physically intact transcripts of large polycistronic messenger RNAs and were applied to 35S avian myeloblastosis virus (AMV) RNA. These conditions do not require the addition of intolerably high concentrations of labeled deoxyribosidetriphosphates. Smaller species of DNA synthesized in the reactions in which full size copies are made do not represent break-down products, but rather DNA anti-complementary to the larger species. This DNA contains, in the proper proportions, all of the sequences expected from the full complement, but it is only 6S in size in alkaline sucrose gradients.

Molecular Basis of Viral Carcinogenesis: A procedure was developed to detect specific virus nucleotide sequences in cellular DNA using the formation of "R-loops." Specific radioactive RNA probes were annealed to a series of DNA fragments under conditions that maximize "R-loop" formation, followed immediately by gel electrophoresis. The DNA fragments homologous to the RNA probe could be clearly identified by autoradiography. Two model systems were explored by this technique. The smallest fragment (Eco-R1 F) of adenovirus-2 DNA obtained by cleavage with endonuclease Eco-R1 is entirely contained within the A fragment generated by Bam-I endonuclease cleavage of Ad-2 DNA and is annealed entirely in the region of the Bam-A fragment, with no RNA migrating with the remaining Bam-DNA fragments.

The second model system was the localization of 28S ribosomal-DNA sequences in an Eco-R1 digest of the nuclear DNA of Xenopus laevis.

At present, the sensitivity of this procedure permits the detection of less than 0.1 ng of DNA with RNA probes labeled to 2×10^7 cpm/ug.

The structure of proviral simian sarcoma virus (SSV-1) genes was examined in their native conformation in chromosomes of the NC-37 human lymphoblast cell lines. From the kinetics of annealing between purified nucleosomal DNA and SiSV cDNA, the concentration of viral genes was found to be identical in nucleosomal and total nuclear DNA, clearly demonstrating that the newly integrated virus DNA was organized in a nucleosomal structure in a manner analogous to that of host DNA. Since the genome was actively synthesizing viral RNA, the data further indicated that transcriptionally active regions of the genome remain associated with histone. However, the nucleosomes over the active genes were organized in a different conformation than nucleosomes formed over transcriptionally inert DNA sequences.

The Purification of Human Reverse Transcriptases from Leukemic Spleens: Leukemic white blood cells and spleens were previously shown to contain particles which encapsulate both a 70S-RNA template and reverse transcriptase. Cores obtained by the proper use of enzymes and nonionic detergents provided a suitably enriched material as a departure point for the purification of the reverse transcriptase. The complete purification of a human enzyme from spleens of patients with chronic lymphocytic leukemia was achieved by the use of DEAE, 'phosphocellulose columns, and agarose gel filtration. The final product consisted of essentially one band in SDS-acrylamide gel electrophoresis, corresponding to a molecular weight of 70,000 daltons.

This enzyme expressed its optimum activity at 37° over a pH range of 7.0-8.2 and exhibited the usual requirements for a divalent ion, with magnesium being the best and manganese serving in a narrow concentration range. In common with the known animal viral transcriptases, the purified human leukemic spleen enzyme could also use DNA, but preferred RNA, as a template. Finally, the ability of the human reverse transcriptases to transcribe a heteropolymeric RNA was demonstrated using AMV-RNA as a template. The product hybridized specifically to AMV-RNA and not to unrelated RNAs.

A similar enzyme has now been isolated in the same stage of purity from spleens of patients with chronic myelogenous leukemia.

Purification of Reverse Transcriptase from Virus-Like Particles Found in Human Breast Cancer: Back hybridization tests of the synthetic DNA product to the template used, and to irrelevant RNA, proved that the human enzyme was acting as a proper reverse transcriptase. Comparison of product complexity by C_0t analysis, using nuclease resistance, showed that the known enzyme was as effective in transcribing AMV-RNA as was AMV reverse transcriptase. Velocity analysis in glycerol gradients indicated that the human enzyme had a sedimentation coefficient corresponding to a molecular weight of 70,000. In common with enzymes from other type B oncornaviruses, it was found that Mn^{++} was a very poor substitute for Mg^{++}.

Preliminary examinations of plasmas from patients with active disease showed clearly that reverse transcriptase could be detected in, and isolated from, such samples. The plasma enzyme appeared to be indistinguishable in size, template responses, and ionic requirements from the breast particle enzyme.

The Search for Virus-Related Proteins by Means of Radioimmunoassay (RIA) in Tissues and Body Fluids: The contractor used modified RIA tests to probe for presence of proteins related to the p30 and gp45 of the simian sarcoma virus in the malignant cells as well as in body fluids of patients with various types of leukemias, with particular emphasis on acute myelogenous leukemia. The results were uniformly negative when they were carried out under conditions that avoid false-positive responses.

Significance to Biomedical Research and the Program of the Institute: A systematic molecular biological study is being pursued to determine the role of viruses in the genesis of human cancer. Studies demonstrated the presence in human cancers of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. New approaches to the study of virus-cell relationships were devised. The contractor developed data which are highly provocative, and his pioneering advances provide a foundation for future investigation leading to an understanding of the virus-cell relationship as it applies to human cancer.

Proposed Course: The contractor will produce intact cDNA transcripts in vitro of the complete of the complete oncornaviral genome, and use these complete copies for restriction enzyme mapping of the viral genome.

The role of nucleoproteins in the regulation of the transcription of oncornaviral genomes will be studied.

The structure of initiation sites of avian oncornaviral RNA will be identified. Diagnostic and/or prognostic techniques will be developed for identifying oncornaviral translation products in body fluids and/or tumor materials of mammalian (including human) species.

The virus-like components of the particles found in human malignancies will be characterized biochemically and/or immunologically, and their relationship to known mammalian oncornaviruses will be determined.

Date Contract Initiated: October 29,1969

Current Annual Level: $1,237,020

DUKE UNIVERSITY (NO1-CP3-3308)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. D. Bolognesi

Project Officer (NCI): Dr. Peter Fischinger

Objectives: (1) Study in detail the properties of structural components of RNA tumor virus and particles, particularly those of mammalian RNA tumor viruses. (2) Use these materials to prepare highly specific antisera which can be applied to analyse cells for the presence of similar virus gene products. (3) Develop appropriate antisera which can be used for detection and identification of tumor virus activities in human malignant cells. (4) Use purified structural virus proteins and the corresponding antisera for possible immunological control of viral disease.

Major Findings:

Immunization Studies in Mice with Friend Leukemia Virus (FLV) Glycoprotein, gp71

Most mouse strains were capable of an immune response (both humoral and cell-mediated) to the FLV gp71. In low virus-inducible mouse strains like STU and BALB/c, there was no ill effects of this immunization, and STU mice could be protected from a challenge with leukemogenic Friend virus following such immunization. In moderate to high virus-inducible mouse strains such as C57BL/6 or AKR, this immunization enhanced activation of endogenous virus; a significantly higher mortality rate was observed in the immunized AKR animals.

Immunotherapy Studies in Mice with Goat Antiserum to FLV gp71

Passive immunization of STU mice with goat anti-FLV gp71 IgG administered up to seven days post-infection, was protective to FLV challenge; no activation of endogenous virus occurred. Type-specific antibodies were also produced by the mice in response to the virus challenge.

Immunotherapy in Feline Leukemia Virus (FeLV)-Infected Cats with Antiserum to Homologous and Interspecies Antigens

Treatment of kittens with goat anti-FLV gp71 serum prevented FeLV infection in a high percentage of animals. The reciprocal protection against FLV infection in mice by treatment with FeLV antiserum was also demonstrated.

Serological Determinants Involved in Cell-Killing In Vitro

A rabbit anti-FLV gp71 serum containing virus group-specific cytotoxic antibodies lysed normal mouse embryo cells. Cytotoxic antibodies involved in interspecies cytotoxicity recognized the interspecies determinant of p30. Antisera against type-specific p12 determinants were also capable of lysis of infected cells.

Serological Determinants of Virion Glycoproteins (gp71) Detectable on Cell Surfaces with Membrane Immunofluorescence and Immunoelectron Microscopy

A. Among murine cells examined, FLV gp71 type-specific cell membrane immunofluorescence was found only on Rauscher murine leukemia virus-producing cells, although group-specificity was present on all murine leukemia virus-producing cells tested. Interspecies cross-reactivity was shared with feline leukemia virus and gibbon ape lymphoma virus-producing cells. Immunoferritin electron microscopy localized these gp71 antigenic determinants on both virus envelope and cell membranes.

B. Using viable-cell membrane immunofluorescence, most established and early passage normal murine cell lines were significantly reactive with rabbit anti-FLV gp71 serum irrespective of their neoplastic transformation, strain genotype, or whether they were of embryonic or adult tissue origin. The only murine cells tested which were not detectably expressing gp71 antigenic determinants were BALB/3T3 lines. An activity discriminating G_{IX+} from G_{IX-} thymocytes was not observed with antisera against either FLV or AKR virus gp71.

C. The surfaces of C3H-, BALB/c-, and C57BL-transformed and normal cells were examined for FLV gp71 and mouse mammary tumor virus (MMTV) gp52 by immunoelectron microscopy. Antiserum to gp71 reacted with all the mouse cells tested regardless of strain, virus production, or state of transformation. A MMTV gp52-like antigen also was detectd on both cell surfaces and virions of C3H and DBA cells.

The Role of Carbohydrate in the Serological and Biological Functions Associated with FLV gp71

Treatment of gp71 with neuraminidase has no detectable effect on gp71 functions. Exposure of gp71 to glycosidases, which resulted in the removal of the majority of the carbohydrate, did not affect interfering and neutralizing antibody-absorbing capacities but did substantially reduce indirect hemagglutination. Preliminary studies showed that infectivity of FLV was not affectd by the carbohydrate removal.

Cross-Reactivity Between Oncornavirus Antigens and Surface Constituents of Human Tumors

Antigens were detected on human myeloid leukemia cells which bear some serological relationship to structural components (principally gp71) of FLV. However, the antigens measured on the human leukemia cells did not bear any clear resemblance to the classical type group or interspecies reactivities of the virus peptides; a minor subdeterminant of gp71 was involved which conceivably could be present on the peptide, but have little or nothing to do with the expression of oncogenic viruses in human leukemic cells.

Several molecular species were precipitated by anti-gp71 antiserum in a lysate of human chronic granulocytic leukemia (CGL) cells. These ranged in molecular weight from 20,000 to 55,000 daltons. The 55,000 molecular weight species was a glycoprotein (gp55). Normal cells or antigen negative cells also yielded materials in immunoprecipitates. Some of these materials coincide with those of antigen positive cells but not with the gp55 moiety.

Antisera prepared against gibbon ape leukemia virus (GaLV) reacted preferentially with lymphoid leukemia cells in contrast to the antiserum to FLV gp71. GaLV blocked the cytotoxic activity of a monkey antiserum to human chronic lymphocytic leukemia (CLL) cells but did not affect the reactivity of a monkey anti-CGL serum on CGL target cells or of anti-FLV gp71 serum on CGL cells.

Significance to Biomedical Research and the Program of the Institute: Although human leukemias or other tumors are not known to be associated with replicating RNA tumor viruses, one cannot exclude the possibility that virus genes exist in human cancer cells and are expressed as discrete antigens on the cell surface in a fashion similar to that in animal cells. Regardless whether or not this occurs, there is considerable evidence that many other aspects, particularly, the immunological consequences of animal and human leukemias, are distinctly related. Therefore, an understanding of the immunological mechanisms in the animal models which are of key importance for host defense, coupled with protocols to artificially stimulate those leading to effective prevention and control of the disease, is of value to reach a better understanding of related events in human cancer. Direct application of the principles which apply in the animal to the disease in humans can occur when appropriate human leukemia specific antigens are identified and characterized, a long-term goal of this research.

Proposed Course: The contractor will determine the immunological response of rodents and cats to exogenous type C oncornavirus infections and use this knowledge to develop immunological preventive and therapeutic measures against oncorna viral-induced leukemia. Surface antigens of human tumor cells which have been found to share some antigenic sites with well-characterized constituents of animal oncornaviruses will be identified and characterized.

Date Contract Initiated: March 1, 1973

Current Annual Level: $481,130

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION (Y01-CP6-0500)

Title: NCI-ERDA Viral Carcinogenesis Program: Regulation of Gene Expression

Contractor's Project Director: Dr. Ray A. Tennant

Project Officer (NCI): Dr. Charles Sherr

Objectives: To study the regulation of tumor virus expression, focusing on molecular mechanisms in induction and repression of tumor virus genomes. Emphasis will be placed on defining the low molecular weight RNA components of viruses and their relation to cellular RNAs.

Major Findings: A new method of isolating intact chromatin in conditions of low ionic strength and with minimal shearing has been adapted to virus-producing AKR embryo fibroblasts. These chromatin preparations contained over 98% of the nuclear DNA and about 80% of the nuclear RNA labeled in a 30 min pulse with (^{3}H)uridine. This RNA remained associated with chromatin throughout various experimental procedures which would be expected to separate chromatin from ribonucleoprotein particles which contain no DNA. These procedures included velocity sedimentation after various degrees of shearing, solubility in low concentrations of $MgCl_2$, and buoyant density determinations. Thus, the RNA was likely to be both nascent and post-transcriptional molecules. presumably in association with the genes from which they were transcribed.

Fractionation of AKR chromatin on steep sucrose gradients yielded two peaks containing most of the DNA and 70% of the pulse-labeled RNA, and a third peak near the top of the gradient containing only 3% of the DNA and 30% of the labeled RNA.

Phenol-extracted fractions of mouse cells with the Fv-1 genotype, Fv-1^{bb} or Fv-1^{nn}, were found to transfer resistance to N- or B-tropic host range types of mouse leukemia viruses of Fv-1^{--} cells (SC-1). Several lines of evidence indicated that the resistance is mediated by cellular RNA. However, purification and characterization of the RNA species and experiments on the mode of action were difficult due to limitations of the assay systems. A standard assay system has been developed to minimize variables. The precision of the plaque assay was ± 20% in repeated assays over several months using single stocks of each virus. Repeated assays of RNA preparations over several months indicated that activity was lost within four-six months, even when the RNA was stored frozen at -140° or in ethanol at -20°C. Statistically significant differences in plaque reduction specific for Fv-1 genotype were seen against the two virus host range types. Most RNA preparations demonstrated specific activity when applied to cells at concentrations of 10-30 ug which corresponds to an estimated 10 (18-20S) RNA molecules per cell. Higher concentrations of RNA gave non-specific plaque reduction, presumably due to an effect on virus attachment similar to the polyanionic effect of polyribonucleotides.

The time course of resistance transfer using RNA indicated that the restriction mediated by RNA inhibits some event occurring within six hours after virus infection. With the development of a sensitive DNA transfection method, it was

possible to examine whether or not Fv-1 gene restriction occurs at steps prior to or beyond proviral DNA integration. When two DNA preparations containing integrated gene sequences of B- and N-tropic murine leukemia viruses, respectively, were inoculated in Fv-1_{nn} (SV-A31 and C57Bl/6) cells, both gave similar efficiency of transfection. Analyses of DNA dose response indicated that DNA transfection of ecotropic murine leukemia viruses followed one-hit kinetics in Fv-1 permissive as well as in Fv-1 nonpermissive recipient cells. Preliminary results indicated that DNA preparations from the Hirt supernatant of newly infected cells behaved the same as the DNA preparations containing the integrated viral gene sequences. These results indicated that the mouse Fv-1 gene restricts leukemia virus infection by interfering with the synthesis of competent proviral DNA in the nonpermissive cells.

A systematic study of DNA transfection led to the following improvements in conditions. The calcium precipitation methods to Graham and van der Eb is very useful for inoculating DNA. Early detection of the DNA transfection of murine leukemia viruses is significantly facilitated by growing DNA-recipient cells in culture medium containing 1 uM hydrocortisone. In transfection with cellular DNA containing integrated sequences, appropriate shearing of the DNA preparation may increase the efficiency of transfection. Optimal DNA sizes are around 25 million daltons. Certain cell lines such as NIH 3T3, SV-A31, and C57Bl/6 mouse embryo derived cells are more susceptible to transfection than other mouse cells tested. No relationship appears to exist between susceptibility to DNA transfection and susceptibility to virion infection in these mouse cells. Procedures were devised which allow detection of virus production in transfected cells by XC assay as early as seven days after infectious DNA inoculation.

Among various associated tRNAs in the 70S RNA isolated from murine leukemia viruses (MuLV), proline tRNAs have been shown to be the most tenaciously bound species. The major isoacceptor $tRNA^{pro}$ of non-infected mouse embryo culture cells labeled with (^{3}H)uridine was isolated in essential purity by employing RPC-5 chromatography. Under hybridization conditions of 0.4 M NaCl buffer solution at 56°C, the purified proline $tRNA_3$. The 35S RNA, with the bound proline $tRNA_3$, regained its template activity for reverse transcription by Rauscher MuLV and avian myeloblastosis virus polymerases. DNA products from this proline tRNA primed reverse transcription were of 4-7S, similar to those synthesized by endogenous reaction using isolated virion preparations of MuLV. The priming activity was also observed in the RPC-5 chromatograph fractions containing proline $tRNA_1$ and $tRNA_2$, suggesting that all three isoaccepting protein tRNAs of mouse cells can serve as a primer for reverse transcription of MuLV genomic RNA.

Limitation of the study with baboon endogenous virus was the quality and quantity of the material available. The Step^1 analysis on one lot of intact 70S RNA indicated that $tRNA^{gly}$ is the candidate primer. This tRNA appeared to have melting temperatures higher than $tRNA^{trp}$-AMV 35S complex.

The 35S RNA of simian sarcoma virus was shown to bind proline tRNA isolated from mouse embryo cells and the complex served as a good template with R-MuLV polymerase.

Cultures of BALB/3T3 (clone A31) cells were treated with 100 ug/ml of IUdR for 24 hours and after ten subcultures, 10^6 cells were found to produce tumors when inoculated subcutaneously in weanling BALB/c nude (athymic) mice.

The viruses produced by the induced A31 cells, designated as BXQ, were found by fluorescent antibody (FA) assay to infect a broad range of cells including mouse, rabbit (CCL-60), mink (CCL-64), and human cells (A_{204}) but did not produce XC plaques in any mouse cells tested. After several passages through mink cells, and three limit dilution isolations, a xenotropic virus was isolated; however, the mink cells also contained a virus which would infect mouse, rabbit, and human cells. When this source of virus was filtered and passaged through Swiss 3T3 cells to exclude the xenotropic virus, the same broad host range and negative plaque pattern persisted. The host range of this apparent amphotropic virus remained stable through several passages in Sc-1, CCL-60, and CCL-64 cells. This virus may be the result of the activation of an endogenous amphotropic type virus, or of a recombination between simultaneously induced endogenous ecotropic and xenotropic viruses. To date the isolated viruses have not produced tumors when inoculated into newborn BALB/c nude mice.

Significance to Biomedical Research and the Program of the Institute: This project is focused on molecular mechanisms involved in viral carcinogenesis. The problem is being investigated in terms of enzymology, immunology, cell biology, and control of gene expression primarily in the mouse leukemia system. The findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: One of the principal objectives of this activity is to define, in molecular terms, the cellular mechanisms by which expression of endogenous RNA tumor virus genomes is regulated. The long range goal is the isolation and characterization of the presumed cellular "repressors" which act in this regulation.

There are two levels of virus-host cell interaction of primary importanance. One is at the genomic level where the critical impact of carcinogenic insult takes place; and the other at the level of the cell membrane where RNA tumor viruses first come into contact with the cell and where major characteristics of neoplastic transformation are expressed. Particular efforts will continue to focus on the host cell factors involved in this virus-cell interaction.

Date Contract Initiated: July 1, 1973 (Separate Viral Oncology contract, September 1, 1972)

Current Annual Level: $409,060

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CP6-1009)

Title: An Immunogenetic Analysis of Mouse Leukemia Viruses

Contractor's Project Director: Dr. Robert C. Nowinski

Project Officer (NCI): Dr. Peter Fischinger

Objectives: To isolate and characterize MuLV produced by AKR leukemias, study expression of endogenous MuLV in high leukemic mouse strains, define genetic control of expression of MuLV, isolate and characterize preleukemic lymphocytes from thymic tissue.

Major Findings: Leukemia cells from mice of the AKR and C58 strains produced leukemia viruses (MuLV) in culture that (1) failed to induced syncytia in the XC plaque assay (XC^-), (2) were poorly infectious for mouse embryo fibroblasts, and (3) yielded low levels of infectious progeny upon establishment of infection. In host range analysis (by measurement of the induction of gs antigens in target cells) these viruses were classified as N-ecotropic. The production of XC^- N-ecotropic MuLV by these leukemia cells was unexpected since mice of the AKR and C58 strains contained high levels of circulating XC^+ MuLV in their sera.

Thymus cells of aged (preleukemic) AKR mice expressed on their cell surfaces elevated levels of antigens associated with MuLV proteins gp70 and p30. Immunoprecipitation of ^{125}I-lactoperoxidase labeled cells with monospecific goat antisera demonstrated that the gp70 antigenicity was contained in a 70,000 dalton protein, wherease the p30 antigenicity was contained in an 85,000 dalton protein. The 85,000 dalton protein has been identified as a glycosylated form of the polyprotein product of the gag gene.

By use of the fluorescence-activated cell sorter the expression of these viral-coded proteins on the cell surface of thymocytes was found to vary both quantitatively with the age of the mouse and qualitatively with the cellular populations that expressed these antigens. Four discrete stages in the leukemogenic pathway were identified: (1) Thymuses of young (two mos) AKR mice contained low numbers of cells that expressed p30 (<1%) and gp70 (2-7%) antigens. The expression of gp70 antigen was restricted to cells in the subcapsular region of the thymus. (2) Thymuses of some six mos AKR mice contain elevated numbers (13-85%) of cells that expressed p30 and gp70 antigens. This quantitative shift in the number of antigen-positive cells also was associated with an increased concentration of viral antigens expressed per unit area of the cell surface. Viral antigens were found on the surface of all cells of the subcapsular region of the thymus, and in variable numbers of cells of the cortical and medullary regions. (3) The thymuses of some eight mos AKR mice demonstrated unilateral hypertrophy of a single thymic lobe. Analysis by light scatter showed that the enlarged lobe contained a population of cells that were larger than the cortical and medullary thymocytes, but which were smaller than leukemic blast cells. This new cell population expressed elevated levels of p30 and gp70 viral antigens. These cells were not leukemic, however, since transfer of high numbers of thesecells to syngeneic hosts did not induce disease. (4) Thymuses of mice with overt leukemia contained large leukemic blast cells. These cells contained extremely high levels of viral antigens on their cell surfaces; and upon transplantation of these cells to syngeneic hosts, they induced rapid disease.

Significance to Biomedical Research and the Program of the Institute: The pathogenic mechanism of viral-induced neoplastic transformation in mice known

to be carrying vertically transmitted oncogenic viruses has been difficult to define. It is important to isolate the influence of various genetic controls on virus expression on target cells and on production of the several viruses during leukemogenesis. Identification of virus-coded marker antigens on cells destined to become neoplastic enable detailed studies on the pathogenesis of "spontaneous" leukemia.

Proposed Course: The following studies will be continued: isolation and characterization of the AKR leukemia virus complex by physical, immunological, and biological techniques; definition of the genetic control of virus expression by use of inbred congenic lines; and isolation and characterization of preleukemic lymphocytes.

Date Contract Initiated: November 1, 1975

Current Annual Level: $90,500

JACKSON LABORATORY (NO1-CP3-3255)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of their Expression

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: Leukemia-prone hairless mice (HRS/J - hr/hr) have significantly higher MuLV titers than the leukemia-resistant nonmutant mice (hr/+ and +/+). This difference was ascribed to the allelic substitution at the hr locus or a closely linked locus; it was greatest (more than 10-fold) at six months to a year of age and coincides with the largest divergence in leukemia incidence among the mutant and nonmutant mice. The humoral response of mutant mice to two antigens (sheep red blood cells and formalin-inactivated, tween-ether disrupted Gross leukemia virus) was equal to that of nonmutant mice; in contrast, delayed type hypersensitivity to both antigens was significantly impaired at 10 weeks of age. Further evidence for impaired T cell function was found in the new AKR/J mutant "streaker" (str) which is allelic with "nude." This mutant lacks a thymus, but has natural immunity to an endogenous xenotropic MuLV. However, the endogenous ecotropic virus titers were significantly higher in homozygous mutants than in heterozygous or nonmutant AKR/J mice. The higher titer may be due to the lack of "killer" T-cells in the hairless mice.

Repeated injections of 1:2 dimethylhydrazine produced mainly colorectal tumors in strains P/J (80%) and SWR/J (60%), whereas AKR/J mice developed leukemias and C57BL/6J mice were resistant to tumor induction. The carcinogenic effects of diethylnitrosamine also depended upon the strain of mouse; strains AKR/J and SWR/J were most susceptible to the development of leukemia and pulmonary tumors, whereas C57BL/6J mice developed liver lesions, including hepatomas and bile duct carcinomas. A single dose of DEN administered to pregnant SWR/J females, mated to AKR/J males, produced tumors only in offspring of mothers injected on days 16 and 18 but not on days 14 and 15 of pregnancy. In contrast, a single injection of diemethylnitrosamine (DMN) produced tumors in (SWR/J x AKR/J)F_1 hybrids only when injected on day 18 of pregnancy. The latter finding suggested that the demethylases and diethylases may be different and separate enzymes which develop at different rates in fetal tissues.

The presence in the growth medium of micromolar amounts of certain steroid hormones enhanced MuLV-specific syncytia formation in the XC test. This enhancement of syncytia formation may be due to an effect on either the mouse cells, the XC cells, or both. Infection of rat tumor SC cells by ecotropic MuLV resulted in the formation "from within" of syncytial plaques 3 to 5 days post-infection, but only if the cells were able to divide several times. Dexamethasone (DXM) enhanced XC cell fusion resulting in larger plaques due to 100-fold increased MuLV production.

Goat anti-AT-124 and anti-RadLV IgG were used against transplanted and chemically induced tumors with the aim of suppressing the virogenes and sarcogenes as well as the virus-coded tumor cell surface antigens. No protection was achieved in several systems. Apparent early enhancement of tumor size occurred, but the effect was transitory. At autopsy the tumors of treated mice were more hemorrhagic and necrotic than those from control animals.

RNA-dependent-DNA polymerase (RDDP) activity associated with particles banding at a density characteristic of oncornaviruses was found in rabbit lymphosarcoma as well as in normal placental and uterine tissues taken during early stages of gestation. The RDDP was distinct from other known cellular DNA polymerases by template-primer preferences, an absolute divalent cation requirement, molecular weight determination and column chromatographic characteristics. Thus, genetic information of a type C RNA viral genome was demonstrated in yet another mammalian species.

Specific anti-tumor effect of RNA isolated from murine tumors and embryos was demonstrated. Unrelated RNA from normal mice of the homologous strain or from heterologous strain was not active. In pregnant females, the preparation was embryo-toxic.

A single intravenous injection of the RNA preparation induced necrosis, hemorrhage, and regression of solid tumors in the strain of origin.

Significance to Biomedical Research and the Program of the Institute: It is now possible, genetically, to ameliorate or eliminate cancer in a mouse throughout its natural lifespan through breeding. By identifying the genes and loci involved in cancer susceptibility and the immunological and physical markers associated with the "high cancer" genes, it is now possible not only to

identify the highly susceptible animal, but to study the biochemical, immunological and metabolic mechanisms controlled by these genes. The development of this information will help (1) elucidate markers to identify the cancer-prone individual; (2) determine how the genes operate in regulating the natural oncogene, with the goal of correcting deficiencies associated with the switching on of cancer cells in susceptibles.

Proposed Course: (1) Further development, characterization, and uses of recombinant inbred lines. (2) Study of the association of the viral group-specific antigen and complete virus with tumor development. (3) Determination of the relationship between genes controlling and influencing the expression of murine leukemia virus. (4) Study of the genetic control of endogenous MuLV expression. (5) Studies with chimeric mice. (6) Study of genetic control of viral-chemical carcinogenesis.

Date Contract Initiated: May 2, 1967

Current Annual Level: $470,000

JOHNS HOPKINS UNIVERSITY (NO1-CP7-1027)

Title: Studies on the Molecular Basis of Viral Carcinogenesis

Contractor's Project Director: Dr. J. Thomas August

Project Officer (NCI): Dr. John Stephenson

Objectives: To isolate and purify structural viral proteins and virus-associated enzymes; characterize their biological, chemical, and immunological properties, and use them as probes for analysis of viral gene expression, in tumor tissues, as agents for possible immunological control of viral infection, and as reagents for detection of virus infection.

Major Findings: This is a new contract; no progress to report.

Significance to Biomedical Research and the Program of the Institute: Specific protein products of virus genetic expression, purified and well characterized, provide the reagents to examine relationships between viruses in terms of gene products and to search for evidence of similar viral gene functions in human disease.

Secondly, these sub-virion proteins provide the means to determine whether effective immunological control of virally-induced disease could be instituted without complication by viral genetic material.

Proposed Course: A major emphasis of Dr. August's research program at Johns Hopkins involves analysis of type C RNA tumor virus coded translational products. These studies include efforts to purify and develop competition immunoassay for a broad range of type C virus structural proteins as well as for the viral coded RNA-dependent DNA polymerase and the putative *sarc* gene product. In addition, experiments have recently been initiated in an attempt

to develop assays for type C virus receptor sites. These assays will be utilized in studies of the molecular structure of RNA tumor viruses and for investigation of virus expression in both normal and neoplastic human tissues. Such studies should provide insight into the possible etiologic role of type C viruses in human cancer.

Date Contract Initiated: May 1, 1977

Current Annual Level: $428,510

LITTON-BIONETICS, INC. (NO1-CP6-1029; successor to NO1-CP3-3211)

Title: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses

Contractor's Project Director: Dr. Marvin Reitz

Project Officer (NCI): Dr. Robert Gallo

Objectives: To characterize virus-like particles in human leukemic cells with respect to DNA polymerase and nucleic acids and characterize and purify viral reverse transcriptases from mammalian viruses, especially primate type C RNA tumor viruses.

Major Findings: Screening leukemic and normal tissue samples for RNA and DNA sequences related to those of the SiSV and BaEV, indicated that some leukemic cell DNA may have viral-related sequences not found in normal tissue's DNA. These sequences hybridized up to 16% of DNA transcripts (cDNA) of SiSV viral RNA and a similar fraction of BaEV cDNA. Human RNA samples revealed only low levels of SiSV-related sequences. A hybridization technique with excess labeled viral RNA was developed to further characterize the proviral DNA sequences.

Type C viral isolates from human tissues were found to be closely related to SiSV by nucleic acid hybridization experiments. Preliminary information also suggested the presence of a component related to BaEV in some instances. Minor differences between the HL23V isolates and other primate type C viruses were also found.

"Helper" virus rescue in vitro using a BaEV-free block of HL23 virus (derived from Bat lung cells) resulted in suggestive evidence of rescue of a BaEV-related component from leukemic cells. The release of reverse transcriptase from HL23 skin fibroblasts into the media after UV induction was also suggested.

Cell strains were established from normal human embryonic tissues and tested for the presence of viral-related information. Data were obtained by immunological tests for the spontaneous or induced expression and transmission of antigens primarily related to BaEV. Some evidence suggested transient release of virus-like particles from selected cultures, but a biologically active virus from human embryonic tissues was not isolated.

A new type C isolate was obtained from a leukemic gibbon and shown to be related to the SiSV-gibbon ape leukemia virus GaLV) group. The tissue distribution of proviral DNA, viral proteins (in collaboration with NCI scientists) and replicating virus was ascertained to be related to infiltration by leukemic cells.

Significance to Biomedical Research and the Program of the Institute: The knowledge acquired is applied to the determination of the etiological relationship of viruses to leukemia in humans, to the development of diagnostic and prognostic modalities for human cancer, and ultimately, to the development of more effective control measures.

Proposed Course: Studies will continue to further define the nature of the virus-like activity expressed in human leukemic cells.

Date Contract Initiated: September 1, 1972

Current Annual Level: $442,783

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP3-3366)

Title: Characterization of Nucleic Acids of the Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Paul C. Zamecnik

Project Officer (NCI): Dr. George Vande Woude

Objectives: To determine the possible molecular mechanisms by which oncogenic viruses change the metabolism of susceptible host cells. To define this system, long-range biochemical studies on the RNAs of AMV were initiated and involve the chemical characterization of the nucleotide structure of viral RNAs and biochemical functions of the viral RNA fractions as measured by their activity in conjunction with RT from the virus and the ability of the 4S RNA fractions to accept amino acids.

Major Findings: During the past year the obstinate difficulties accompanying use of the reverse transcriptase procedure for sequencing the region of 35S RSV and AMV RNAs just internal to the poly(A) terminus were overcome. Primers consisting of $d(pT)_9pdG$, $d(pT)_9prG$, and $d(pT)_9pdGrA$ were synthesized. With these primers it was possible to copy the terminal residues at the 3' ends of RSV and AMV, with the oligo $d(pT_9prG$, for example, lining up correctly at the internal end of the poly(A) tail.

Large-scale polymerization reactions were carried out with purified RSV 35S RNA, purified reverse transcriptase, the above primer, and various combinations of two or three deoxynucleoside 5'-triphosphates, one of which carried ^{32}P-label in the α-position. Under these conditions, the $d(pT)_9prG$ primer was slightly extended by addition of only 1-2 kinds of deoxynucleotide residues. Alkaline digestion released the $d(pT)_9$ tail and made it possible to analyze the sequences synthesized by nearest neighbor analysis.

In this way, the determination of a tetradecadeoxynucleotide sequence immediately adjacent to the 3'-terminal poly(A) segment of RSV 35S RNA was made. The sequence was as follows:

5'--------- (G)CCAUUUUACCAUU$(C)_n$-polyA-3'. The (G) was inferred, and n varies from 1-4.

The corresponding sequence for AMV 35S RNA appeared to be similar but not identical.

Significance to Biomedical Research and the Program of the Institute: This project was initiated in the expectation that elucidation of the nucleotide sequences of the 70S RNA of AMV and possibly other oncogenic RNA viruses might reveal segments active in specific functions and as binding sites of viral polymerases or inhibitors, thereby increasing our knowledge of transcription processes.

Proposed Course: This contract terminated September 30, 1976. Work on sequencing of the 3' end of RSV 35S RNA will continue under contract NO1-CP7-1007).

Date Contract Initiated: June 29, 1971

Current Annual Level: $140,000

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP7-1007)

Title: Sequencing of the 3' End of RSV 35S RNA

Contractor's Project Director: Dr. Paul Zamecnik

Project Officer (NCI): Dr. Charles Sherr

Objectives: By means of oligonucleotide primer-initiated template copying of purified 35S RNA from both cloned RSV (Prague strain, subgroup C) and AMV (BAI strain A) with purified reverse transcriptase from AMV, the contractor will (1) determine the sequence of 100 or more nucleotides adjacent to the poly(A) sequence at the 3' terminus of oncornavirus 35S RNA and (2) determine the complete nucleotide sequence at the 5' end of AMV 35S RNA from the site of initiation by endogenous primer to the 5' terminus of the viral genome (approximately 100 nucleotides).

Major Findings: This is a new contract. No progress has been reported to date.

Significance to Biomedical Research and the Program of the Institute: The 3' terminus of 35S RNA of oncornaviruses almost certainly plays an important role in viral integration and replication. The endogenous primer molecules are now known to bind with 35S RNA subunits within 150 nucleotides of the 5' terminus; furthermore, preliminary evidence indicates that circularization of the viral genome occurs during an early stage of replication. Recent preliminary evidence suggests that some portion of the 3' terminal sequence is reiterated at the 5' end. These results suggest that one key to understanding the mechanism of replication of RNA tumor viruses involves determination of the nucleotide sequences at both the 5' terminus and the 3' region adjacent to the terminal poly(A) segment of the viral genome. Such sequence analysis may provide insight into the structural basis for formation of circular intermediates during replication. Ultimately, knowledge of the primary structure at both the 5' and 3' ends of the genome may indicate mechanisms for formation of a provirus structure and integration of the provirus into the host cell genome.

Proposed Course: Sequencing of 35S RNA from the 3' end will continue at least 100 nucleotides.

Date Contract Initiated: January 1, 1977

Current Annual Level: $82,500

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP5-3562)

Title: Studies on the Leukemia Virus DNA Polymerase and Terminal Deoxynucleotidyl Transferase

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. Edward Scolnick

Objectives: (1) Carry out a detailed analysis of DNA polymerases from RNA tumor virus particles and from normal and neoplastic cells. (2) Study the mechanism of double-stranded DNA synthesis by the avian myeloblastosis virus DNA polymerase and the functions of the subunits of the enzyme in infected cells and variation in DNA polymerases in various stages of cell growth.

Major Findings: A fluorescent antibody test for terminal transferase (TdT) was developed to detect enzyme-containing cells. It afforded a routine assay for leukemias containing terminal transferase as well as a tool to study the normal human, mouse and calf cells that contain the enzyme. It also was used to study the biosynthesis of terminal transferase. A 60,000 dalton polypeptide was found that could be a precursor to the enzyme (which, from calves, has 2 subunits: 28,000 and 8,000 daltons). Mouse cells containing terminal transferase were classified into three groups: thymus cells, bone marrow cells that are in the "prothymocyte" lineage, and bone marrow cells that have characteristics of "pre-B" lymphocytes.

Studies on reverse transcriptase were concentrated on synthesis of the longest products during the endogenous reaction in virions of murine RNA tumor viruses. Synthesis of up to 10% of the product as apparently full-length transcripts was achieved by using carefully defined conditions. Some of the DNA made in the absence of actinomycin was active in transfection to mouse cells and led to synthesis of virus that was, in limited tests, indistinguishable from the Moloney virus used to make the DNA. In the continuing study of the translation of Moloney virion RNA, preliminary evidence was found for a 180,000 dalton protein product that could be a limited product of the gag and pol gene products.

Significance to Biomedical Research and the Program of the Institute: Fundamental studies on oncogenic virus replication are necessary to define the role of these viruses in malignant cell transformation. In vitro synthesis of infectious nucleic acid and in vitro translation of proteins allows first level mechanistic studies which can logically be expected to provide means for controlling the infections and/or transforming processes.

Proposed Course: Conditions will be optimized to produce and analyze infectious DNA. Analysis of genetic control of viral replication will be analyzed. Mechanism of DNA proviral synthesis by reverse transcriptase and the function of RNAse H will be explored. In vitro translation will be used to determine precursor sequences and cleavage sites.

The significance to TdT to lymphoid cell differentiation will be investigated. Studies on human leukemia ALL cell TdT will be determined.

Date Contract Initiated: May 1, 1971

Current Annual Level: $170,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP5-3519)

Title: Isolation and Characterization of Type C RNA Tumor Viruses and Diagnostic Testing and Service Functions

Contractor's Project Director: Dr. M. Lee Vernon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To define the events, with special emphasis on the role of endogenous type C RNA viral genomes, associated with activation of oncornaviruses in non-producer cell cultures by chemicals, DNA viruses, or spontaneously; and to characterize the endogenous viral isolates.

Major Findings: Rat epithelial cells infected with Kirsten murine sarcoma virus (KiMSV) produced three classes of transformed cells: virus producer, non-producer (NP), and sarcoma-positive, leukemia-negative (S+L-) cells. The transformed cells produced fibrosarcomas in newborn rats and formed larger cell aggregates than those formed by normal counterpart cells when suspended in liquid above an agar base. Revertants of NP human

osteosarcoma cells originally transformed by KiMSV have been isolated after incubation at 40.5°C. MSV could no longer be rescued from the morphologic cell revertants obtained at elevated temperature, and the growth properties were similar to those of the nontransformed parent human osteosarcoma cell line. The revertants were not tumorigenic in nude mice. The revertants showed an enhanced sensitivity to MSV superinfection. KiMSV-human osteosarcoma cells treated with BUdR transiently released type C particles and reverse transcriptase (RT) activity; the induced virus was not infectious. Human osteosarcoma (HOS) cells transformed by KiMSV or n-methyl nitrosoguanidine (MNNG) grew in the aggregate form above an agar base, produced colonies in soft agar, had fibrinolytic activity, and produced tumors in nude mice. When transplanted into antithymocyte-serum (ATS)-treated hamsters, the cells transformed by MNNG (0.01 ug/ml) or KiMSV produced tumors; but MNNG (0.01 ug/ml) transformed cells did not. Control HOS cells did not grow in the aggregate form but formed colonies in soft agar, had low fibrinolytic activity and did not form tumors in nude mice or ATS-treated hamsters. One control clone had high fibrinolytic activity and did not produce tumors in nude mice and ATS-treated hamsters.

Oncorna-like viruses (guinea pig retravirus) have been induced with halogenated pyrimidines in a variety of guinea pig tissues and have now been found to be released from a strain 13 guinea pig tumor, induced by KiMSV, after long-term culture. The virus was non-infectous, had Mg^{++} preference in RT assays, did not share group-specific antigens with known type C viruses, and was antigenically distinct from murine and cat type C viruses. A guinea pig herpesvirus (GPHV) was isolated from guinea pig cells after cocultivation of BZ L*C and GH L_2C cells with mink cells. In contrast to human herpesvirus, the GPHV had a relatively narrow host range, but showed antigenic relatedness to the human herpes simplex virus.

Preliminary evidence indicated that there was significant prevention or suppression of polyoma virus transformation in rat cells treated with antibody to type specific endogenous rat leukemia virus (RALV) but not with non-specific Rauscher leukemia virus (RLV) antibody. Current studies of tumors of rats produced by DNA viruses and RNA tumor viruses confirmed and extended earlier observations on the existence of a tumor antigen common to all rat tumors.

The cellular aggregation assay was analyzed and compared to cell growth in soft agar as an indicator of the tumorigenic potential of cultured cells with the conclusion that this was a fast, accurate and reproducible means of evaluating *in vitro* transformation. Proliferation of cells in the aggregate form was considered to be more indicative of tumorigenic potential than aggregate size differences alone.

Two hundred eighty-one samples were examined for the presence of virus as a service to this and other contracts authorized by the Project Officer. The digallic acid mordanting procedure was used in a comparative study to determine its usefulness in delineating virus morphology. As an adjunct to routine procedures it was very helpful in distinguishing true spikes as found in type B particles. A comparative study of oncorna-like virus (Retravirus) in various guinea pig tissues revealed that while there were quantitative differences, morphologically identical particles could be induced in several normal guinea

pig tissues treated with BRdU in leukemic guinea pig tissues and in a tumor spontaneously releasing virus. Comparisons to type B and type C particles using routine procedures, digallic acid mordanting procedures and negative stains indicated that the guinea pig retravirus was sufficiently different to justify a separate classification. Natural history studies in C3H/A^{V}y mice revealed no significant difference in the incidence of type C particles between male and female mice. Type B particles were found only in female mammary tumors.

Three hundred fifty RT assays were performed as a service to this and other collaborative programs.

Serodiagnostic tests for infection by common murine viruses were conducted on 107 sera. Unsuccessful efforts were made to isolate Sendai virus from 21 mouse lung extracts in tissue culture and 2 mouse lung extracts using mouse virus isolation techniques. H-1 and KRV were found in 2 of 23 rat tumor lines tested by the RAP test. No contaminating viruses were found in 3 mouse tumor lines by the MAP test.

Significance to Biomedical Research and the Program of the Institute: The isolation of human type C RNA viruses, and the definition of events associated with expression of virus or viral products would be the major handle for controlling cancer, either through the use of preventive vaccines or by interruption of the processes leading to virus expression and neoplasia.

This contract has high priority in terms of its probability of contributing to the NCI objectives. The professional staff are among the most sophisticated in the area of tissue culture and virus rescue, and the service aspects of the program have been performed in a superior manner and have made important contributions in their own right.

Proposed Course: Continue studies outlined in above Objectives Section.

Date Contract Initiated: November 23, 1974

Current Annual Level: $600,000

MINNESOTA, UNIVERSITY OF (NO1-CP6-1055)

Title: Transcriptional Regulation of Eukaryotic Gene Sequences

Contractor's Project Director: Dr. Anthony J. Faras

Project Officer (NCI): Dr. Edward Scolnick

Objectives: (1) To delineate the control mechanisms involved in expression of RNA tumor virus genes in eukaryotic cells by performing studies on avian tumor virus genes in transformed and revertant vole cells in vitro; (2) to determine how the transformed phenotype is maintained; (3) to investigate the possible role of translation, polyadenylation, methylation, mRNA transport and processing in the post-transcriptional regulation of tumor virus gene expression.

Major Findings: In an effort to gain an understanding of viral-induced oncogenesis, the control mechanisms involved in the regulation of RNA tumor virus genes were studied; particular emphasis was placed on regulation of the genes responsible for malignant transformation in eukaryotic cells. The cell type used for these studies was a field vole cell line transformed by an avian oncornavirus. One of the interesting features of these transformed vole cells is their ability to revert to the normal phenotype. It was demonstrated that these revertant vole cells (1) exhibit all of the growth properties of uninfected, normal vole cells; (2) contain the entire virus genome, including the gene for malignant transformation; and (3) express all of the virus genes, including the transforming gene sequences, as RNA at levels similar to the transformed cells from which they were derived. Further, the entire transforming gene was shown to be present and biologically active in revertant subclones, indicating that the phenomenon of reversion in this cell system is not due to a deletion or point mutation in the transforming gene sequence, rendering it inactive. Thus, in contrast to all of the other known oncornavirus-transformed/revertant cell systems studied to date, the vole cell system is unique in that it exerted post-transcriptional regulation of not only viral structural genes but the transforming gene sequences as well.

Significance to Biomedical Research and the Program of the Institute: In an effort to gain an understanding of virus-induced oncogenesis, delineation of the control mechanisms involved in the regulation of RNA tumor virus genes, particularly those responsible for malignant transformation in eukaryotic cells, is essential. From the studies on transforming and revertant vole cells, conclusive evidence has been obtained indicating that the control of virus gene expression and virus-induced oncogenesis occurs at the post-transcriptional level in this cell system. Since virtually nothing is known regarding the mechanisms by which eukaryotic cells regulate the expression of their own genes, let alone oncornavirus genes at this level, investigation of transformed and revertant vole cells may elucidate the post-transcriptional factors that influence the genetic expression of transforming gene sequences in eukaryotic cells. Elucidation of the cellular and viral functions involved in these regulatory phenomena is likely to provide insight into the regulation of oncogenic viruses in eukaryotic cells and quite possibly contribute to an understanding of the mechanisms on oncogenesis in general. Furthermore, these studies may shed light on the mechanisms by which the exogenous infection of cells by RNA tumor viruses can, in some instances, result in the establishment of nonexpressed inherited viral genomes exemplified by the presence of endogenous viruses in normal cells.

Proposed Course: The contractor will further investigate the transformed/revertant vole cell system in an effort to elucidate the post-transcriptional factors that ultimately influence the genetic expression of transforming gene sequences in eukaryotic cells.

Date Contract Initiated: June 30, 1976

Current Annual Level: $62,999

NEW ENGLAND MEDICAL CENTER (NO1-CP6-1046)

Title: Immunology of Murine Leukemia Virus Infection

Contractor's Project Director: Dr. Robert Schwartz

Project Officer (NCI): Dr. John Stephenson

Objectives: To determine (1) influence of the H-2 genetic region on the immune response of mice to murine leukemia virus (MuLV). Is there Ir-gene effect? (2) Correlation between genetically determined immunological responsiveness to MuLV and ability to eliminate MuLV. (3) Immunological responsiveness to MuLV administered across the tropism barrier. (4) Correlation between genetically determined immune response to MuLV and incidence of neoplasms. (5) Role of the complement system, if any, in elimination of MuLV. (6) Immunological and virologic correlates of the RgV-1 gene, the H-2 locus which specifies resistance or susceptibility to leukemia induced by several types of MuLV.

Major Findings: The early phase of infection by an endogenous MuLV was studied in several strains of mice. Two new genetic influences that restrict infection by MuLV were discovered. One of these is located within the H-2 complex and specifies dominant susceptibility to B-tropic MuLV. The other is not linked to the H-2 region and specified dominant resistance to B-tropic MuLV.

Results of preliminary experiments indicated that an intact T-cell system is required for expression of the H-2-determined resistance to B-tropic MuLV. Antibodies to viral envelope antigens did not seem to be protective *in vivo*.

An *in vitro* method was developed to elucidate the mechanism of immune-resistance to MuLV. Early results with this system suggested that *in vivo* phenomena relevant to infection by MuLV can be reproduced entirely *in vitro*.

A possible regulator gene that controls spontaneous expression of B-tropic MuLV was discovered in congenic mice.

Significance for Biomedical Research and the Program of the Institute: This program can help elucidate immunological and genetic markers predisposing to cancer, contributing to early detection of risk. Since cancer is at best difficult to treat and cure, early detection and prevention are of major importance.

Proposed Course: The mechanism of age dependence of immunoresistance to MuLV will be investigated. Resistance of C57L mice and their crosses to MuLV will be analyzed. Genetically-determined immune responses to purified viral proteins will be analyzed in detail. Correlations between expression of ecotropic and xenotropic viruses in immunosuppressed mice developing a reticulum cell sarcoma will be determined.

Date Contract Initiated: June 30, 1976

Current Annual Level: $62,488

SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE (NO1-CP6-1049)

Title: Studies on the Mechanism of Oncogenesis by RNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Edward Scolnick

Objectives: (1) Analyze human cancers for DNA sequences specific for RNA tumor viruses and for unique DNA polymerase activity. (2) Investigate the biological role of intracellular 20S viral RNA and its possible processing from viral 35S RNA. (3) Conduct studies on the identification and isolation of virus-coded transforming proteins specified by murine oncornaviruses.

Major Findings: Basic studies on RNA tumor virus replication were supported only during the first 5 months of 1976. Evidence was obtained that the 80,000 molecular weight mammalian type C virus reverse transcriptase can be cleaved in vitro to a 30,000 dalton fragment that contains only RNase H activity. It was shown that the β subunit of the αβ form of avian myeloblastosis virus (AMV) reverse transcriptase was responsible for binding to $tRNA^{Trp}$, the primer for in vitro proviral DNA synthesis. The enzymatically active α subunit did not form a tight complex with $tRNA^{Trp}$ under numerous experimental conditions. A double-stranded RNA-specific endonuclease purified from murine cell microsomes was shown to cleave 35S Moloney murine leukemia virus (M-MLV) RNA into 2 distinct fragments. M-MLV genomic RNA has 15-23 internal N^6-methyladenylic acid residues and a 5'-methylated cap sequence of m^7 $GpppG^mpCp$. A protein synthesis requirement was demonstrated at 2-3 hr after infection with Moloney murine sarcoma-leukemia virus, a time when 50% of parental RNA was bound to polyribosomes in an EDTA releasable form.

The following results were obtained in studies with human tissues and cultured cells. This work was supported during the entire contract year: (1) A new DNA polymerase that uniquely responds to poly 2'-O-methylcytidylate·oligodeoxyguanylate (poly(Cm)·oligo(dG)) was identified in a number of human malignant cell lines and in a leukemic spleen; this DNA polymerase may be specific for malignant cells and could be a candidate human reverse transcriptase. The enzymes were purified from a human melanoma cell line and some of their physical and chemical properties were determined. (2) Reverse transcriptase-containing particles were not detected in (a) culture media and cells from 10 different chronic myelogenous leukemia bone marrow samples cultured in conditioned medium, (b) media from cultures of 5 different indicator cell lines cocultivated with 11 different leukemia whole blood samples, (c) fresh lymphocytes from 3 lupus erythyematosus patients and spleens from 2 other patients. (3) The appropriate assay conditions with poly(Cm)·oligo(dG) were established for viral reverse transcriptase from a variety of viruses. (4) A double-stranded DNA probe representative of the gibbon ape leukemia virus genome with a molecular complexity of 4.9×10^6 daltons which was synthesized on the viral RNA template by purified AMV αβ DNA polymerase, was used to measure proviral copy numbers of 4 and 15 in an infected human cell line and in a gibbon ape lymphosarcoma cell line, respectively.

Significance to Biomedical Research and the Program of the Institute: This research program is aimed at understanding, in detail, the mechanism of cell transformation by RNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer. Basic research on the molecular biology of normal and virus-infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis, and for developing a rational therapy for viral diseases and cancer.

Proposed Course: The new DNA polymerase Cm will be purified and characterized, and its significance to transformation will be investigated. The relationship between this enzyme and particulate reverse transcriptase activity will be determined. Analysis of human normal and neoplastic tissue will be conducted for nucleic acid sequences specific for subhuman primate RNA tumor viruses.

Date Contract Initiated: June 1, 1976

Current Annual Level: $270,000

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP4-3375)

Title: Immunologic Study of Type C RNA Tumor Virus

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This program is comprised of three integrated segments aimed at (1) identification of the various virion and non-virion-associated products of endogenous type C virus genomes in mice, (2) quantitation of the spontaneous immune responses of mice to the products of their endogenous type C virus genomes and any immunopathologic consequences thereof, and (3) modification of expression of endogenous type C virus genomes by immunization with virus or purified virus antigen vaccines or by immunosuppression.

Major Findings: Quantiative and biochemical studies were continued on the endogenously expressed murine leukemia virus (MuLV) gp70s in a number of murine strains. Expression of gp70 was found to be restricted to certain anatomical sites and cell types, among which lymphoid and epithelial cells were prominent. On a quantitative basis, the major site of gp70 expression was the male genital tract.

During fetal development, gp70 first appeared in the hematopoietic liver of 14-day old embryos and by day 18, it was already expressed at anatomical sites similar to those of the adult. In toto, these results showed that control of expression of the MuLV genome in adult and developing mice was linked to differentiation.

A number of gp70 proteins were isolated from purified virus as well as from sera and secretions of mice. A tryptic analysis of the peptides was done. In this way, the structure of a virion protein could be compared to its

endogenously expressed counterpart. The structure of the proteins from AKR and NZB mouse sera were compared to the Rauscher, SLV, AKR, NZB xenotropic, NIH xenotropic, BALB/c xenotropic, and NZW xenotropic viruses. It appeared that the endogenously expressed protein molecules resembled the gp70 of the xenotropic viruses, especially of the NZB type. More than one type of gp70 could be expressed in a single strain of mice depending on the anatomical site studied. This observation indicates that different endogenous viral genomes can be expressed at different sites of cell differentiation.

The immunopathologic consequences of oncornavirus expression were studied. An important advance was the characterization of two new strains of mice which develop a fatal lupus-like syndrome early in life. These mice become important in the search for common immunologic or virologic determinants of immunopathologic disease.

Human serum, but no serum from a number of other species, was observed to inactivate and lyse oncornaviruses from a number of different sources. Lysis occurred in the absence of antibody. A detailed analysis of the role of the human complement system in mediating this lytic process indicated that human C1q, in the absence of immunoglobin, interacts directly with oncornaviruses. Binding of C1 via C1q in this manner led to activation of C1r, C1s and thus of the classical complement pathway. Integrity of the classical pathway was an absolute requirement for lysis, although activation of the alternative pathway considerably amplified the amount of lysis obtained, possibly through involvement of the C3b-dependent feedback mechanism. Activation of complement was accompanied by deposition of complements on the virus surface and lysis occurred on completion of the complement reaction sequence. Thus, in this system, the C1q subunit of C1 subserved a specific recognition function normally associated with antibody. This ability of human serum to inactivate oncornaviruses may represent a natural defense mechanism operative in vivo which deters expression of intact oncornaviruses in human malignancies.

Significance to Biomedical Research and the Program of the Institute: This program is relevant to the goals of the National Cancer Institute in relation to determinantion of cause, early detection of risk, and eventual effective prevention of cancer. The etiological association between type C RNA viruses and cancer has been firmly established in chickens, mice, hamsters, rats, and cats, and is strongly implicated in cows, gibbon apes, woolly monkeys and baboons as well. In addition, particles have been observed in human placentas in numbers suggesting that they are universally present. Expression of this apparently universal oncogenic potential, however, is dependent on the immunologic responses of the host. Although these immunologic controls are well recognized, the mechanisms are poorly understood. Characterization of the viruses, virus antigens and host cell responses will provide important insights into the etiological role of the viruses under varying immunologic conditions, and the means of detecting and interfering with their oncogenic properties through the use of viral vaccines.

Proposed Course: The contractor will continue ongoing studies, as follows: (1) Characterization of the products of endogenous type C viral genomes and their interaction with host immunologic defense mechanisms. (2) Analysis of the molecular nature of the viral genome products, and definition of the

relationship of their production to host genetic or developmental events, including cancer. (3) Definition of the spontaneous immune responses of mice to their endogenous type C oncornaviruses and any associated immunopathologic events. (4) Attempts to manipulate natural production and control of endogenous type C viruses or non-virion-associated viral molecules to determine immunopathologic complications, effect on control of natural cancers and/or normal growth and development. (5) Definition of the potentially important direct neutralizing effect of primate complement on type C viruses and associated neoplastic events.

Date Contract Initiated: June 29,1972

Current Annual Level: $553,000

SIDNEY FARBER CANCER CENTER (NO1-CP5-3539)

Title: Attempt to Isolate Type C Virus from Cultured Human Leukemia Cells

Contractor's Project Director: Dr. David M. Livingston

Project Officer (NCI): Dr. George Todaro

Objectives: To attempt to isolate an intact type C virus from freshly-cultured human leukemia cells, either by finding such cells which spontaneously produce virus, or by inducing virus production by physical, chemical or immunological means.

Major Findings: Rabbit anti-human Fab immunoadsorbents were used to remove populations of normal B lymphocytes from relatively large aliquots of Ficoll-Hypaque purified leukemic white cells from both marrow and peripheral blood. Standardization experiments showed that established T cell lines passed through the column with 99% efficiency while Ig^+ human B cell lines adhered to the column with 60-90% efficiency in the first pass and greater than 95% efficiency in in the second pass.

Superior growth of leukemic cell cultures was obtained using reduced concentrations of oxygen. The success rat at which bona fide leukemia cell lines are established may be substantially increased by culture conditions combining T-cell purification with reduced oxygen tension.

Attempts to "supertransform" leukemic cells with simian virus 40 (SV40) were not successful. Thus far, SV40 infection of cells yielded no advantage in the establishment of cell lines.

In an effort to identify, unequivocally, the phenotype of an established cell line and to determine if it was the same as that of the leukemic cells in the specimen from which it was derived, anti-sera were developed which were highly specific for various types of normal and malignant human hematopoietic cells. The antigens identified with these sera have never before been recognized in human cells. Their recognition offers significant new opportunities to identify, purify, establish lines and study specifically differentiated forms of both

normal and transformed human hematopoietic cells. Presently available antisera prepared by this technique are capable of identifying specific subclasses of T-cells, myeloid cells and, possibly, leukemia-specific antigens. The new antigens defined by this procedure are:

Th_1 - Present on 90% of thymocytes and fetal thymocytes but only on 50-60% of normal peripheral blood T-cells in all populations of fresh leukemic cells from acute lymphocytic leukemia (ALL) patients and all T-cell lines tested. Th_1^+ cells proliferate in mixed lymphocyte reactions but do not respond to soluble antigens by ^{3}H-TdR incorporation.

BK - Present on T-cell leukemias and T-cell lines, not on peripheral blood T-cells.

AB 312 - Present on T-cells of acute leukemia origin and T-cell lines. Absent from peripheral blood T-cells, thymocytes (adult and fetal), "null" cell leukemic cells, acute myelogenous leukemia (AML) cells, red cells, B-cells, B-cell lines, PHA and MLC stimulated T-cells. One possibility is that the antigen recognized is specific to leukemia cells of thymic origin.

p23,30 - A pure pair of proteins which exist on the surfaces of normal B-cells, some leukemia myeloblasts, "null" cells but not on T-cells, thymocytes, T-cell leukemias. It exists on the surface of all "null" cell acute lymphoblastic leukemias tested to date.

Two cell lines (LAZ 292, 213) were established from the peripheral blood buffy coat of a patient presenting with a diagnosis of ALL of the T-cell variety. The cells in culture are small and grow as a monodisperse non-adherent culture. These cells have many characteristics of T-cells of leukemia origin, as well as other properties not usually associated with normal peripheral T-cell and not found in other T-cell lines. In particular, the rosetting properties of LAZ 191 are not typical of normal T-cells or thymocytes or even of 4 human T-cell lines believed to be of leukemia origin. They consistently form both E-rosettes and EAC-rosettes. Analysis of uncultured, Ficoll-Hypaque-purified leukemia cells from this patient demonstrated that they also formed both E- and EAC-rosettes. The ability to form both types of rosettes appears to be the property of a single, homogeneous population rather than a mixture of T- and B-cells existing in the same culture. Further studies showed that LAZ 191 will form EA-rosettes, indicating the presence of Fc receptors on the cells. LAZ 191 cells are negative for surface immunoglobulin, surface lysozyme (a myeloid cell marker) and p23,30. On the other hand, the cells carry Th_1 and BK, two antigens which are T-cell specific. LAZ 191 and the patient's uncultivated, original leukemia cells also react strongly with Ab 312. Tests for Epstein-Barr virus nuclear antigen (EBNA) were repeatedly negative. Thus, LAZ 191 is an abnormal (Fc^{++}, Ab 312) T-cell (E^+, Th_1, BK) line which represents, in culture, the patient's leukemia cells and is a bone fide leukemia cell line of established heritage.

LAZ 221: This cell line was established from the peripheral blood of a patient presenting with ALL. The cells are small and grow as a monodisperse, non-adherent culture. The cells do not form either E- or EAC-rosettes. The pattern of antigenic activities (BK^-, Th_1^-, IG^-, $p23,30^+$) is consistent with that found in specimens of blood from approximately 85-90% of patients with ALL in the Western Hemisphere (so-called "null cell" leukemia). In addition, this cell line is EBNA negative. Thus, LAZ 221 is a continuous line of leukemia cells from a patient with the most prevalent form of ALL.

LAZ 61: This cell line, derived from a pediatric patient with a diagnosis of AML, is Ig^+ and $EBNA^+$ but contains significant amounts of lysozyme (supposedly a myeloid cell or histiocytic marker). Sequential fluorescent activated cell sorter analyses demonstrated that the same cells which are Ig^+ are lysozyme positive, indicating that the culture is not composed of two cell types. In contrast, four B lymphoid lines, derived fromn normal individuals, and one B cell line derived from an AML patient, contain no detectable quantities of lysozyme. The consistently elevated lysozyme in LAZ 61 cells and its absence in other lines indicates that LAZ 61 may possibly have a myeloid heritage.

Nine lines were tested for arginine deprivation induction of 70S RNA and RNA-dependent DNA-polymerases on several occasions. No reproducible appearance of polymerase containing particles in 200-400 fold concentrated supernatants could be detected.

Cocultivation for up to 3 months with a standard xeno- and homogenic cell set, without evidence of the persistent production of polymerase producing particles, has been completed for cell lines from 6 patients with rheumatoid arthritis and from 7 patients undergoing renal allograft rejection. Nearly all cell lines which were established during the first 2 years of the contract have undergone 3 months of cocultivation with negative results.

A method was developed which permits one to readily detect, under conditions of low, non-specific background binding, 0.1 or less copies of integrated proviral DNA sequences in infected cells. Specificity controls indicate that under conditions in which the test is carried out, the results are highly specific to the virus under consideration.

Twenty-four specimens of DNA extracted from human leukemia cell packs were examined by hybridization with excess RNA on filters; two DNAs from individuals with acute myelogenous leukemia revealed a significant degree of hybridization with woolly monkey virus RNA.

Bone marrow cells of murine origin, transformed by Abelson virus, were found to be cystine auxotrophs.

Significance to Biomedical Research and the Program of the Institute: The successful isolation of a type C virus from human leukemia cells would permit the development of reagents to determine whether such viruses are natural etiological agents of this human cancer. For example, the ^3H-DNA product of the endogenous reverse transcriptase reaction of a human type C virus would be the ideal probe for the investigation of the partial genetic expression of such viruses in nonproductive, normal and malignant human cells.

The antibody to the reverse transcriptse and gs antigen and other viral specific proteins of such a particle would be superb tools for the development of analytical systems designed to detect type C viral proteins in non-productive human cells. The identification of a viral-specific transformation protein in transformed, but not normal cell, would be the strongest evidence that endogenous type C virus is involved in the etiology and pathogenesis of human neoplasia.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: April 24, 1974

Current Annual Level: $140,000

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1003)

Title: Influence of Virus-Related Genes on Susceptibility to Cancer

Contractor's Project Director: Dr. Edward A. Boyse

Project Officer (NCI): Dr. Stephen O'Brien

Objectives: (1) The breeding, maintenance, and supply of congenic strains of mice which differ from their partner strains in expression of genes concerned with production or control of leukemia virus, and (2) testing these paired strains for their susceptibility to spontaneous and induced malignancy.

Major Findings: This is a new research effort. No progress reports have been required as yet.

Significance for Biomedical Research and the Program of the Institute: Genetic control of susceptibility to spontaneous and viral-induced leukemia in mice has been well documented. However, the mechanism of control by the several loci involved has only recently received attention. The present study has been designed to assign specific functions to each controlling genetic allele. Identification of genetic control mechanisms in murine strains should form the groundwork for identifying similar controlling factors in other species, including man.

Proposed Course: "Quartets" of four strains, representing two base strains and two corresponding congenic strains in which the differentiating alleles have been switched both ways, will be developed and supplied to all qualified interested investigators. Seven quartets are planned covering the H-2, Fv-1, PC, TL and G_{IX} loci. The influence of each of the alleles on expression of virus, host response to viral products, and occurrence of leukemia will be investigated by monitoring virological, immunological and pathological parameters during the life span of these strains.

Date Contract Initiated: December 15, 1976

Current Annual Level: $119,247

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP5-3518)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. John Stephenson

Objectives: (1) To isolate temperature-sensitive (ts) mutants in genes coding for virion proteins of avian sarcoma viruses. (2) To isolate ts transformation mutants from avían RNA tumor viruses which differ in origin and pathogenicity from RSV or B77. (3) To isolate nonconditional deletion mutants of avian RNA tumor viruses.

Major Findings: The isolation of ts mutants from avian erythroblastosis virus, avian myelocytoma virus (MC29) and the Mill Hill strain of Murray-Begg endothelioma virus was begun. Focus assays were adapted to the study of these viruses, and stocks of mutagenized virus were prepared. Initial testing of cells in foci, produced under permissive conditions for mutants, was begun.

A new type of transformation defective avian sarcoma virus was obtained from the Schmidt-Ruppin strain of Rous sarcoma virus. This transformation defective deletion mutant appears to have retained part of the src gene. It had been characterized biochemically and was used to map avian sarcoma virus ts mutants.

Avian erythroblastosis virus, avian myelocytoma virus (MC29), and the Mill Hill strain of Murray-Begg endothelioma virus are being tested for the presence of their replication defective agents. MC29 nonproducer cell lines were isolated and their defect in the synthesis of viral structural proteins is being characterized. Similar studies are in progress with the Mill Hill strain 2 virus and with avian erythroblastosis virus.

A new temperature-sensitive replication mutant LA83 was isolated and characterized physiologically. The mutation appeared to be a late one; preliminary data suggested it may affect the function of the DNA polymerase.

A large number of temperature-sensitive avian sarcoma viruses which have a lesion in the src gene have been tested in downshift experiments. Transformation, which normally occurs upon shift from the nonpermissive down to the permissive temperature, previously was reported to require protein synthesis, suggesting that a transforming protein produced by sarcoma viruses is inactivated irreversibly under nonpermissive conditions in temperature-sensitive mutants, and that it has to be synthesized de novo. The purpose of screening large numbers of temperature-sensitive mutants in src for a requirement of protein synthesis in retransformation upon shift to the permissive condition was to uncover any mutant in which the inactivation of the putative transforming protein at the nonpermissive temperature is reversible and in which retransformation could occur without protein synthesis. No such mutants were found among 37 isolates tested, suggesting that reversibly temperature-sensitive transforming proteins are rare.

Several new coordinate mutants which neither transform nor replicate at the nonpermissive temperature were mapped by three factor crosses. The mutations appeared to be located in the pol gene toward the 3' end from the previously described pol mutation LA337.

LA337 was mapped by a three-factor cross and found to be located toward the 5' end of the pol gene.

Significance to Biomedical Research and the Program of the Institute: The work performed by this contractor has provided valuable genetic tools for study of oncornavirus gene functions in replication and transformation and location on the viral genome. Future production of additional mutants from all segments of the genetic map will provide valuable tools for isolation and characterization of those gene functions relating specifically to potential viral carcinogenesis.

Proposed Course: This contract will continue to isolate new mutants of avian RNA tumor viruses.

Date Contract Initiated: October 15, 1971

Current Annual Level: $160,748

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP4-3214)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. R. L. Heberling

Project Officer (NCI): Dr. Alfred Hellman

Objectives: To study the viral flora in placentas and embryos of primates with emphasis on type C viruses.

Major Findings: The pathogenicity of the baboon endogenous virus (BaEV) was determined in nonhuman primates and in beagle dogs. BaEV infected dogs when inoculated in utero or at birth, but no pathology resulted from this infection. Similarly, marmosets, cebus monkeys, baboons, and chimpanzees were unaffected by BaEV inoculation. Fibrosarcomas were induced by a BaEV pseudotype of Kirsten murine sarcoma virus [MSV(BaEV)] in dogs, marmosets, cynomolgus monkeys, and chimpanzees, but not in cebus or squirrel monkeys. One baboon inoculated intracerebrally developed a brain tumor and metastatic lung tumors, all fibrosarcomas. All the species inoculated with BaEV or MSV(BaEV), except the baboon, developed neutralizing (SN) antibody against BaEV. The baboon showed a partial tolerance to BaEV by not developing SN antibodies, but did develop a rise in precipitating antibodies (RIP) following inoculation. Cell-mediated immune (CMI) responses were also observed in MSV(BaEV)-infected animals.

Autogenous CMI to BaEV, as measured by lymphocyte blastogenesis in the presence of viral angigen, was demonstrated in adult male and female baboons. These initial studies indicated a greater response in male baboons. Attempts at in vivo rescue of the Kirsten murine sarcoma genome in dogs were not successful.

A squirrel monkey RNA virus (SMRV), with the properties of a type D virus has been characterized. The morphology of SMRV resembles Mason-Pfizer monkey virus (MPMV), and the virus DNA polymerase, like that of MPMV, preferred magnesium ions over manganese ions, and a low KCl concentration for optimum activity. The SMRV polymerase had an optimum pH of 7.5 compared to 8.1 for MPMV. A 70S RNA was shown by the simultaneous detection assay. The SMRV polymerase was partially neutralized by MPMV antiserum but no other antigenic relationships were detected between these viruses. SMRV also was unrelated to other mammalian oncornaviruses (MLV, FeLV, RD114, BaEV, SSAV, and GALV) when tested by immunodiffusion.

Similar SMRV isolates were recovered from various tissues of four adult, five newborn, and two fetal squirrel monkeys. Virus particles with eccentric nucleoids resembling type C viruses were seen in the placenta but not in other fetal or adult tissues. Nucleic acid hybridization and host range studies indicated SMRV is an endogenous, xenotropic virus of squirrel monkeys.

SMRV was inoculated into squirrel monkeys, marmosets, baboons, and beagles but no overt pathology resulted. Virus was not recovered from beagles that were inoculated at birth and sacrificed 1 to 2 months later.

Attempts to isolate virus from a human leukemia patient's placenta. a rhesus monkey-baboon hybrid placenta, and tissues from a leukemic chimpanzee were not successful.

Significance to Biomedical Research and the Program of the Institute: Evidence was obtained for the existence of vertically transmitted type C viruses in "normal" primate tissue. Further study is required to determine whether these agents have any direct relationship to the development of malignancy in any primate.

Proposed Course: Efforts will be continued to determine the function of these virus particulates in the primate host.

Date Contract Initiated: June 3, 1971

Current Annual Level: $350,000

TEXAS, UNIVERSITY OF (NO1-CP6-1017)

TITLE: Biosynthesis of Oncorna Proteins in Mouse and Human Cells

Contractor's Project Director: Dr. Ralph B. Arlinghaus

Project Officer (NCI): Dr. Takis Papas

Objectives: To study the synthesis and processing of murine leukemia virus proteins in mouse and human cells.

Major Findings: Biochemical and immunological evidence indicated that the core proteins (p30, p15, p12 and p10) of Rauscher murine leukemia virus are

synthesized by way of an intracellular polyprotein precursor of about 200,000 daltons molecular weight (Pr1a+b) that is processed in stages to yield intermediate polyprotein precursors with approximate molecular weights of 80,000 daltons, 67,000 daltons, 60,000 daltons and 40,000 daltons, termed Pr3 to Pr6, respectively.

Preliminary results indicated that the order of proteins in the 67,000 dalton precursor (Pr4) is NH (p12, p30). p15.p10 - COOH.

The intracellular precursor to the major viral glycoprotein, gp69/71, was identified as two closely migrating glycopolypeptides (Pr2a+b, ~90,000 daltons) lacking fucose.

Two non-glycosylated virion proteins (p15E and p12E) were found to be structurally related to the intracellular viral glycoprotein precursor (Pr2a+b).

Murine sarcoma virus-transformed cells were shown to contain a 60,000 mol. wt. polypeptide precursor to murine virus p30. This p60 precursor was found in all ml-clones of Moloney sarcoma virus-transformed cells examined but not in uninfected cells, and was either absent or present in small amounts in leukemia virus-infected cells.

Some inhibitors of proteolytic enzymes, such as TPCK (tolylsulfonyl-phenylalanyl chloromethyl ketone, an α-chymotrypsin inhibitor), interferred with the processing of precursor polyproteins Pr1a+b (≃200,000-d) and the "gag" precursors Pr3 and Pr4 (80,000-d and 70,000-d, respectively.

The 200,000-d precursor polyproteins Pr1a+b were shown to contain antigenic determinants of the reverse transcriptase and each of the four "gag" proteins. This component has been termed "gag-pol" precursor.

Intermediate precursors of the 70,000-d virion reverse transcriptase were identified by use of antisera specific for the virion reverse transcriptase. These intermediate precursors had molecular weights of about 145,000-d, 135,000-d and 85,000-d. They were termed PrRT1, PrRT2 and PrRT3, respectively. PrRT3 appeared to be the smallest RT-specific protein present in infected cells.

Evidence was obtained for the apparent non-covalent association between RT precursors and the "gag" prtoein, p30.

The amino acid analogue of arginine, canavanine, inhibited the cleavage of "gag-pol" Pr1a+b (≃200,000) and "gag" Pr3 (≃80,000).

The "gag" precursors and "env" prevursor were made in Rauscher leukemia virus infected cells in amounts 15 to 20 times that of "pol" precursors. The "gag-pol" precurosr Pr1a+b (200,000-d) was made in amounts similar to the "pol" precursors PrRT1, PrRT2 and PrRT3.

A model has been proposed which fits these results and results of others. This model involved translational control in which the 35S genomic RNA is visualized as having one initiation site for ribosomes near its 5' terminus.

After translating the "gag" precursor Pr3 (≈80,000-d), ribosomes are frequently released from the viral message. However, a few ribosomes (1 of 15 or 20) escape termination and proceed through the "pol" gene, terminating and producing the "gag-pol" precursor Pr1a+b (≈200,000-d). The "env" genes are translated from a subset messenger RNA which represents the 3' one-third section of the 35S virus RNA. Envelope genes could also arise from an internal initiation site within the genomic RNA. Based on results of other viral systems, this latter initiation site would be non-functional in intact viral RNA but would become active in a subset messenger RNA. Drugs which interfere with the synthesis and processing of Rauscher leukemia virus genomic RNA selectively decreased the cleavage of one particular viral precursor polyprotein, the 70,000-d "gag" precursor Pr4. A model depicting the interaction of viral RNA Pr4 has been proposed. Such interaction is likely to result from a direct affinity between Pr4 and virus RNA. In this model, a "procapsid" structure, consisting of subunits composed of uncleaved Pr4, is formed before interaction with virus RNA. The formation of this structure is determined only by the specificity of the interactions of the protein subunits. The Pr4 components p10 and p12, which probably interact with the viral RNA, are depicted on the inside and the other proteins, namely p30 and p15, are on the outside of the structure. Entrance of the viral RNA into this structure results in neutralization of the positive charges of the basic p10 in the center allowing the formation of a more compact structure which, in turn, may catalyze the proteolytic cleavage of Pr4. Cleavage of Pr4 can occur in particles lacking viral RNA, but at a slow rate. Such particles also lack RNA of any kind.

Significance to Biomedical Research and the Program of the Institute: Oncornaviruses cause some types of cancer in many different animal species. By inference, one may assume that a viral etiology also exists for some types of cancer in man. In animal cells, viral information is often incomplete or incompletely expressed so that virus particles may not be assembled in some tissues. Similarly, in some human cancers, oncogenic viral information may be only partially expressed, accounting for the difficulty in establishing evidence for a viral etiology of human cancer. An immunological probe could serve as a basis to develop a clinical assay for oncogenic viral gene products in human tissues. Furthermore, the information obtained in this study is expected to further the knowledge of the relationship of these viral-specific gene products in the transformation process.

Proposed Course: The overall objective of this project is to prepare a suitable immunological probe to detect oncornavirus proteins in human cells. The specific aims include the study of the synthesis and processing of the Rauscher murine leukemia virus proteins, produced in mouse and human cells by the use of antisera prepared against components of disrupted virus particles. The antisera will be used as probes to compare, contrast and characterize the intracellular, viral-specific polypeptides synthesized in virus-infected human and mouse cells. Antisera prepared against disrupted human cell-adapted Rauscher virus, candidate human tumor viruses, and simian sarcoma virus will be used as immunological probes to detect oncornavirus gene products in human cells.

Date Contract Initiated: January 1, 1976

Current Annual Level: $135,468

2. Office of Program Resources and Logistics

Dr. Jack Gruber, Chief, OPR&L, VOP, DCCP
Dr. Garrett V. Keefer, Staff Scientist, OPR&L, VOP, DCCP
Dr. Lea I. Sekely, Staff Scientist, OPR&L, VOP, DCCP

AMERICAN TYPE CULTURE COLLECTION (NO1-CP-6-1047)

Title: Curatorial Preservation and Development of Reference-Grade Tumor Viruses

Contractor's Project Directors: Dr. Jerome Kern
Dr. Charles D. Aldrich

Project Officers (NCI): Dr. David Howell
Dr. Garrett Keefer

Objectives: To biologically characterize and historically trace the origin of selected groups of tumor viruses, including avian, murine, feline, and primate, in order to develop and obtain reference-grade tumor virus materials. To serve as an archival repository for seed stocks of important virus materials from the Viral Oncology Program. To provide documented histories and characterizations of materials which have been provided in quantity to NCI collaborating investigators.

Major Findings: Virus stocks supplied by NCI are being characterized biologically, biochemically, and biophysically. The infectivity of reference lots of oncogenic viruses is being determined in cultured cells in vitro by a minimum of two parameters. Biochemical analysis of virion-associated RNA-dependent DNA polymerase activity is carried out on reference lots, and requirements for optimal activity of the enzyme are being determined. Other parameters and procedures will also be used for the complete characterization of reference-grade viruses. These include analysis of virion composition in terms of proteins, glycoproteins, and RNA, and the immunological properties and specificity of reference viruses. Quantities of reference lots of oncogenic viruses are stored at -70°C, and small seed lots will be preserved at -196°C.

Significance to Biomedical Research and the Program of the Institute: The necessity for this type of project is underscored by the fact that very extensive research has been carried on throughout the world with virus materials supplied by the Office of Program Resources and Logistics of the Viral Oncology Program. It is therefore of primary importance that highly characterized reference stocks of these viruses be prepared and be available for retrospective studies which will certainly be necessary as new techniques and research concepts occur in the future.

Proposed Course: During the next year the contractor expects to complete in vitro studies and initiate immunological and biophysical experiments with RLV, RSV (Pr-C), and FeLV(R): initiate biological and biochemical

studies with Mouse Mammary Tumor Virus; and start testing concentrated preparations of oncornaviruses for the presence of xenotropic contaminants.

Date Contract Initiated: June 15, 1976

Current Annual Level: $80,000

CALIFORNIA, UNIVERSITY OF (NO1-CP-3-3237) and
NAVAL BIOSCIENCES LABORATORY (YO1-CP-4-0201)

Title: Development and Evaluation of Cell Substrates for Utilization in Cancer Research, and NBL Support Facility

Contractor's Project Directors: Dr. Stewart Madin
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officers (NCI): Dr. James Duff
Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biosciences Laboratory (NBL), Oakland, California. The program of the CCL is funded by a contract (NO1-CP-3-3237) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBL by an interagency transfer of funds (YO1-CP-4-0201) between NCI and NBL. This project includes the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic applied research.

Major Findings: During the 15 months reporting period, the contractor continued to initiate, grow, preserve, characterize, and distribute a variety of human and animal cells for utilization in cancer research. Procedures utilized are such that antibiotic-free cultivation is achieved in the majority of cases. Routine laboratory procedures are such that mycoplasma contamination occurs at only a very low frequency among the numerous cultures initiated, grown, and preserved in this laboratory.

During the reporting period, over 1,250 cell culture seed stocks were distributed to approximately 200 individual scientists in over 200 separate shipments. Since the last report, over 50 new cell lines have been produced involving human cancer material, normal human specimens, and some from other animals. Selective trypsinization methods permitted the growth of epithelial cell lines from about 40% of the tumor tissues. A number of valuable cell lines of tumor origin have been received from other laboratories and processed at CCL for distribution; among these were 14 of human origin. Approximately 50 laboratories have submitted over 175 individual cell cultures for analysis of species and cell line purity. Of these more than 130 could be studied. Almost one-fourth of the cell

lines submitted proved to be HeLa contaminants; others were inter-species contaminants of a variety of animal cells.

Morphologically abnormal cell lines are being compared to morphologically normal cells in terms of growth pattern, cell doubling time, saturation density, clonal growth on various substrates, karyology, and tumorgenicity in nude mice. Clonal growth studies are in progress on a variety of low passage, nonHeLa, human cancer cell lines in an effort to define the optimum nutrition and environmental conditions for culture of such cells.

Techniques for isoenzyme analysis by acrylamide gel electrophoresis have been improved and used to assay for glucose 6-phosphate dehydrogenase (G6PD), 6-phosphogluconic dehydrogenase (6PGD), and alkaline phosphatase isoenzymes in a variety of cell structures. A commerical preparative-scale electrophoresis apparatus is being investigated with a view to possible performance improvement and its subsequent utilization in the examination of growth factors associated with various tissue culture media components.

Karyologic characterization of all cells maintained in the repository continues and specific collaborative programs requiring the use of karyologic data are in progress.

Significance to Biomedical Research and the Program of the Institute: The contractor has an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and VCP contract laboratories. The contract continues to develop techniques for the identification and study of tumor cells oriented toward a study of the fundamental biology of tumor cells and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; continue a reference laboratory for karyology of cells in culture; continue applied research in the biology of cell cultures.

Date Contract Initiated: October 1, 1962

Current Annual Level: $550,000 and $75,000

CHILD RESEARCH CENTER OF MICHIGAN (NO1-CP-3-3333)

Title: Inter- and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Directors: Dr. C. S. Stulberg
Dr. W. Peterson

Project Officer (NCI): Dr. David McB. Howell

Objectives: This contract provides Viral Oncology Program collaborating investigators with a service for rapid establishment or confirmation of species identity of cell culture systems.

Major Findings: During this report period approximately 324 cultures were examined by 600 separate tests that utilized genetic markers of three general kinds, i.e. species antigens, isozymes, and chromosomes. Other markers were employed as required. Results were reported to submitting investigators usually within ten days after receipt.

Approximately 20% of the cell cultures submitted were in non-concordance with the species designated, had more than one species of cells present in the culture, or had an intraspecies characteristic different from that of the purported source. Cell species encountered included: human, marmoset, rhesus monkey, cercopithicus monkey, mouse, rat, cat, dog, hamster, rabbit, cow, horse, mink, baboon, gibbon, and orangutan.

Significance to Biomedical Research and the Program of the Institute: In the search for oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Generally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolator systems are all dependent upon the assurance of the identity of the cell cultures used.

Proposed Course: The contractor will continue to provide service to Viral Oncology and its contractors by rapidly determining interspecies and intraspecies identity of cell culture systems. Additionally, the contractor will extend and develop marker systems in accordance with needs for identification.

Date Contract Initiated: June 26, 1973

Current Annual Level: $115,000

CHILDREN'S HOSPITAL MEDICAL CENTER (NO1-CP-6-1036)

Title: Supply of Pediatric Oncology Specimens

Contractor's Project Director: Dr. Gordon F. Vawter

Project Officers (NCI): Dr. Lea I. Sekely
Dr. Paul Peebles

Objectives: To supply fresh and/or frozen pediatric tissue specimens for distribution to and study by collaborating cancer research laboratories.

Major Findings: The Children's Hospital Medical Center is a 343-bed, general pediatric hospital with active pediatric oncology units in surgery,

radiation therapy, and medical oncology. The hospital's pathology department provides the diagnostic surgical and autopsy services to these units and to pediatric oncology patients of the Sidney Farber Cancer Center. During the current report period necessary supplies and equipment have been purchased and several areas of responsibility concerning the collection and distribution of tissue specimens were assigned. Approximately 70 specimens of tumor and related tissue from surgery and 30 similar specimens from autopsy have been provided to the NCI repository for subsequent distribution to collaborating investigators.

Significance to Biomedical Research and the Program of the Institute: The supply of specimens from pediatric patients with malignant disorders is necessary to provide investigators in cancer research with material needed to carry out experiments to determine the possible viral etiology of cancer.

Proposed Course: To continue to carry out the activities described above.

Date Contract Initiated: March 10, 1976

Current Annual Level: $28,363

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-5-3531)

Title: Biological Resources Management Information System

Contractor's Project Director: Mr. James Getschman

Project Officers (NCI): Ms. Wilma Varrato
Ms. K. Hancock

Objectives: To implement a computerized central inventory and management system for the various resources of the Viral Oncology Program.

Major Findings: During the last year, the contractor continued to provide computer support to the Office of Program Resources and Logistics. The central inventory system was adapted to utilize an interactive input program and a file structure which detects and allows for the correction of data errors at the time of input. Programming support was provided for maintenance of the satellite subsystems. The contractor provided data handling support for continuous maintenance of data files. In addition, the contractor collected data for a system of inventory for the resources accrued by the Laboratory of RNA Tumor Viruses, VOP.

Significance to Biomedical Research and the Program of the Institute: A detailed information system of availability, storage location, clinical, demographic and other pertinent data is required to facilitate the shipment of research resource materials needed by collaborating cancer research scientists.

Proposed Course: With the completion of scheduled activities, this contract was terminated on February 28, 1977. The support activities identified above are now performed by another contract.

Date Contract Initiated: May 3, 1971

Current Annual Level: $292,849

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-7-1000)

Title: Support Effort for the Coordinated Processing of Fresh and Viable Human Specimen Materials

Contractor's Project Director: Ms. Gretchen Stroemer

Project Officers (NCI): Dr. Lea Sekely
Ms. Wilma Varrato

Objectives: The primary objective of this project is to assist the Office of Program Resources and Logistics (OPR&L), Viral Oncology Program, in the pick-up, handling, coordination, and delivery of human neoplastic and normal specimens for cancer research.

Major Findings: Biological materials handled by this project are available as by-product material from necessary clinical diagnostic and treatment procedures. These include tumor tissues, blood samples and other surgical and autopsy tissues contributed from resource contractors in the United States and Canada. During the current period the contractor provided service-support to OPR&L to help coordinate the distribution of large numbers of human specimens, supplied by NCI contract sources, thus permitting recipient research investigators to undertake intensive studies on the possible viral etiology of human cancer.

Significance to Biomedical Research and the Program of the Institute: It is not sufficient that sources of human tumor materials for cancer research exist; it is also necessary that the capabilities of these sources be coordinated with the needs of investigators who require specific, and often fresh, specimens. This contractor, under the direction of the NCI project officer, serves as a direct interface between the user and supplier.

Proposed Course: This contractor will specifically assist OPR&L in the pick-up, handling, and delivery of human neoplastic and normal specimens. These human specimen materials will be provided to investigators at the direction of NCI.

Date Contract Initiated: October 8, 1976

Current Annual Level: $149,973

EG&G/MASON RESEARCH INSTITUTE (NO1-CP-7-1001)

Title: Computer Support Effort for OPR&L Resources Management

Contractor's Project Director: Mr. James Getschman

Project Officers (NCI): Ms. Wilma Varrato
Ms. Kathryn Hancock

Objectives: To assist in processing, storage, and retrieval of data associated with research resource materials of the Viral Oncology Program.

Major Findings: This is a new contract effort that was initiated on March 1, 1977. It is anticipated that the contractor will function well in assisting the Office of Program Resources and Logistics in maintaining information on availability and storage location of various research resource materials at production laboratories and/or repositories.

Significance to Biomedical Research and the Program of the Institute: Computerization of resources data makes it possible for the NCI Office of Program Resources and Logistics to exercise close control over the inventory of viruses, sera, human tissues, and other materials provided by the VOP and used in cancer research. In addition, computerization makes it possible to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby, permitting rapid response to the needs of the Viral Oncology Program while avoiding resource excesses or shortages.

Proposed Course: The contractor will provide systems analysis, computer programming, and management of data as required by OPR&L. The contractor will extend and refine the central inventory system as necessary to meet the ever-changing requirements for materials used in cancer research. Appropriate management reports will be generated as required by the Viral Oncology Program. Maintenance of implemented subsystems will continue. Work on new subsystems will be scheduled by OPR&L as needed.

Date Contract Initiated: March 1, 1977

Current Annual Level: $265,682

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-2-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officers (NCI): Dr. David McB. Howell
Dr. Garrett V. Keefer

Objectives: To provide a service related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans. Production and quality control involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During this report period the contractor processed 9,622 equivalent liters of virus-containing fluids harvested from several tissue culture systems and, as directed by the Project Officer, distributed the purified virus concentrates, cells, and special vaccine preparations to the Office of Program Resources and Logistics repository and to individual investigators involved in a variety of research projects. The flexibility of the program was emphasized in several ways: The high-volume production and purification of AKR leukemia virus was terminated and the production of Moloney leukemia virus in the dense NIH-3T3 cell line was increased. Five xenotropic viruses were produced in quantity from cell lines received from an NCI investigator - BALB:virus-1, BALB:virus-2, NZB:MuLV, NIH:MuLV (ATS-124).

The xenotropic viruses now comprise about half of the contractors total required production volume. The cell lines yielding xenotropic virus only produce about one-tenth as much virus, compared to the exogenous viruses. Therefore, considerably increased virus-tissue culture fluid volumes are required to maintain equivalent quantities of concentrated xenotropic virus for the various recipient investigators.

The quality of the contractor's virus products is frequently monitored by gel electrophoretic analysis of viral RNA (the spectrophotometer scanning of the gel detects approximately one microgram of RNA) and by PAGE analysis for the viral structural protein profile and envelope glycoprotein content. As part of the effort to continually increase the quantity and improve the quality of available viruses, the contractor has compared the yield of virus and the RDDP activity from two cell lines releasing endogenous NZB virus (received from Dr. F. Dixon and from Dr. J. Levy) to the current producing cell line: NZB mouse virus/human A673 cells (Kirsten pseudotype). The contractor has selected and scaled-up production of the NZB mouse embryo, clone 35, endogenous virus of Dr. J. Levy.

Last year in cooperation with Dr. F. Dixon and Dr. A. Essner of Scripps Clinic, the contractor established a purification procedure which yields increased quantities of GP 69/71 from AKR virus. This procedure requires a freshly-harvested virus fluid, immediate centrifugation in a glycerol density gradient, pelleting and resuspension; no freezing until after the virus pellet is resuspended. This procedure has also been successfully applied to BALB:virus-2 and to NZB:MuLV xenotropic viruses.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract helps meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and also serves to find the best means of producing

and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: The contractor will continue to provide AKR virus, Gross leukemia virus, Moloney leukemia virus, and special vaccine preparations; and will provide other special preparations as requested by the NCI project officer. The contractor will continue to develop and optimize methods for producing the highest virus yield with consistantly high biological qualities, with the flexibility to quickly accommodate shifts in Program requirements.

Date Contract Initiated: March 27, 1972

Current Annual Level: $650,000

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-4-3334)

Title: Virus Processing and Production Facility

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officers (NCI): Dr. George Vande Woude
Dr. Jack Gruber

Objectives: The purpose of this contract is to establish and maintain a facility capable of a variety of batch-scale production of animal oncogenic and/or suspected oncogenic viruses; and also for the processing of large volumes of virus containing fluids supplied by other NCI-designated laboratories or investigators.

Major Findings: During this report period the contractor continued to grow relatively large volumes of various Herpes viruses (HSV-1: Mal, H5RD, H44, KOS, Patton, and Synsytial V; HSV-2: Rabson, 233, and Savage) in Vero cells, and Herpesvirus saimiri in OMK. The contractor continued utilizing the scaled up procedure for polyethylene glycol (PEG) precipitation/Renografin gradient centrifugal purification of the Herpes viruses, and processed, radio-labelled (when requested), and characterized 2,029 equivalent liters of virus fluids by the PEG/Renografin technique. Additional Herpes virus was recovered from 1,325 gms. of packed cells using a Freon-113 extraction-centrifugation procedure.

The contractor also grew, processed, and characterized many different viruses, including baboon placenta virus; MSV (FeLV); C-type murine xenotropic virus from BALB/c and NIH lines; Moloney leukemia virus; gibbon ape lymphoma virus, Thai strain; guinea pig embryo, 3B11IC-MSV, and 19 other RNA viruses from the research laboratories of NCI. Six thousand, six hundred and ninety-two liters of virus-containing fluids were concentrated and purified by conventional, sucrose-gradient centrifugal techniques.

In addition, the contractor received virus-containing tissue culture fluids including Kirsten leukemia virus, Moloney leukemia virus, guinea pig embryo,

MSV-FeLV, and baboon inguinal lymph node virus from various VOP collaborating investigators. A total of 2,265 liters of virus-containing fluids, received from other laboratories were concentrated and purified by sucrose gradient centrifugation methods.

Significance to Biomedical Research and the Program of the Institute: This project provides cooperating investigators with the opportunity to process large quantities of virus-containing tissue culture fluids, as well as smaller quantities of a variety of viral agents of special interest. There is a strong need for this effort within the Program since it specifically relates to the determination of the possible viral etiology of human cancer.

Proposed Course: The work described above will be continued at approximately the same level of effort, and be flexible enough to support the changing needs of the Viral Oncology Program and its collaborating investigators.

Date Contract Initiated: June 15, 1974

Current Annual Level: $575,000

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NO1-CP-3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officers (NCI): Dr. Garrett Keefer
Dr. Lea I. Sekely

Objectives: To monitor the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to VOP collaborators for transplantation, tissue culture and virus isolation studies.

Major Findings: During this report period, a group of 70 aging and/or irradiated rhesus monkeys and progeny were monitored by daily observations and tri-annual physical and hematologic evaluation. These examinations specifically concentrated on the detection of developing neoplasms. During the most recent survey, 9 animals were noted to be moderately emaciated and one has shown severe progressive emaciation. Two animals have large masses that have been characterized as a lipoma and a benign fibrious tissue tumor; five animals have a slight to moderate enlargement of the uterus; two animals have a very large mass in the lower abdomen in the region of the uterus; one animal continues to have a palpable mass in the region of the liver; and skin and/or subcutaneous nodules or masses were present in 13 animals.

Seven deaths occurred in the colony during this report period. Five of the 7 adult animals (71%) that died had tumors. Neoplasms encountered in the 5 animals included a renal cell carcinoma, a pituitary adenoma, an ovarian carcinoma, an adrenal tumor, and one animal had both a carcinoma of the

ileo-cecal region and a bronchogenic carcinoma. Specimens from the renal carcinoma and the adrenal tumor were shipped to VOP collaborating investigators.

The nine mature young females added to the colony several years ago for breeding purposes have been transferred to another effort at Yerkes Regional Primate Research Center and are no longer supported by this contract.

Significance to Biomedical Research and the Program of the Institute: The VOP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provides tumor tissues and other important specimens from aging, irradiated sub-human primates for research within the VCP. At the same time the contractor conducts a screening operation for the appearance of virus-like particles or viral antigens in the monkeys. Malignant changes in these irradiated primates may provide useful information which might be applied to humans, who are also subjected to similar physical stresses.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor using light and electron-microscopy. Specimens of these tumors will be made available to VCP investigators. In addition, a program is underway to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

Current Annual Level: $28,000

EMORY UNIVERSITY, SCHOOL OF MEDICINE (NO1-CP-6-1051)

Title: Supply of Human Tumor Specimens and Related Material for Cancer Research

Contractor's Project Director: Dr. Douglas R. Murray

Project Officers (NCI): Dr. Lea I. Sekely
Ms. Wilma L. Varrato

Objectives: To supply by-product surgical human mammary tissue and serum to NCI for cancer research utilization.

Major Findings: The contractor is located at one of the largest hospital units in the southern part of the United States, and has access to a large number of neoplastic mammary tissues and sera. During the current period, the contractor made 85 shipments to the NCI resources processing center and NCI repository. Specimens supplied consisted of 110 neoplastic breast tissues and 75 sera.

Significance to Biomedical Research and the Program of the Institute: Finding of virus-like particles as well as 70S RNA and reverse transcriptase in breast cancer tissue by several investigators within VOP strongly suggest that a virus may be associated with human breast cancer, therefore, breast neoplastic specimens are of critical importance in studies of the possible viral etiology of human mammary cancer.

Proposed Course: To carry out the activities described above.

Date Contract Initiated: June 28, 1976

Current Annual Level: $37,430

ENVIRO CONTROL, INC. (NO1-CP-6-1021)

Title: Monitoring of Biohazard Containment Facilities

Contractor's Project Director: Dr. Robert W. McKinney

Project Officer (NCI): Dr. David L. West

Objective: In collaboration with the Viral Oncology Office of Biohazard Safety (OBS), NCI, the contractor: performs safety inspections of virus production facilities which provide resources to NCI to determine compliance with relevant safety standards; performs biological safety and environmental control surveys to contract research facilities for the purpose of providing guidance in biohazard control technology with aim of reducing accident potential and promoting the integrity of experimental systems by protecting them from contamination; evaluates the effectiveness and accomplishments of previous OBS safety program and recommends means to improve the OBS control program; provides safety, environmental microbiology, and engineering consultation to NCI laboratories and to other parties seeking assistance from OBS; and procures and installs essential safety equipment needed by Program scientists involved in cancer research. Through requests from the Office of Research Safety, OD, NCI, the contractor also assists other parties by providing technical safety information and guidance.

Major Findings: During this period the contractor's efforts have been directed toward: a) conducting biological safety and environmental control surveys at VCP contractor laboratories; b) assessing and improving the designs of prototype safety equipment developed for Program researchers; c) development of design criteria and engineering details to assist cancer researchers in obtaining quality and reliable research facilities when renovations or construction projects are undertaken; d) formulating recommendations for OBS consideration on major issues of research safety; e) continuance of support of NCI safety and environmental control program through generation and dissemination of information; f) providing technical consultation on research safety matters to parties seeking assistance; g) providing engineering design and review services.

At the request of the Office of Research Safety, OD, NCI, the contractor has provided consultation. training, and facility planning services to NIH for its programs in recombinant DNA research.

Significance to Biomedical Research and the Program of the Institute: This contract contributes biological safety and environmental control expertise to the VOP and to the general scientific community. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an important support to the NCI Office of Biohazard Safety.

Proposed Course: The contractor will continue to provide technical assistance to the VCP contractors on problems of environmental control, personnel safety, and product protection. The contractor will participate in approximately 15 safety and environmental control surveys. The contractor will review all plans and specifications for renovation and new facility construction projects upon request. All recommendations will be based on compliance with accepted engineering design, biological safety and environmental control practices.

Date Contract Initiated: September 29, 1976

Current Annual Level: $233,000

FLOW LABORATORIES, INC. (NO1-CP-5-3566)

Title: Animal Holding Facility to Support Intramural Research on RNA Tumor Viruses

Contractor's Project Director: Ms. Judith T. Snow

Project Officers (NCI): Dr. John W. Pearson
Dr. Garrett V. Keefer

Objectives: To support NCI intramural research activities being conducted in the Viral Immunotherapy Section, Laboratory of RNA Tumor Viruses. The nature of the intramural activities requires the long-term holding of aged mice, rats, and guinea pigs for the study of the effects of drug therapy, surgery, vaccines, non-specific immune stimulators and interferon used alone or in combination against viral-associated transplantable tumor lines.

Major Findings: During this report period the contractor received and held a total of 4,400 rats and guinea pigs. These animals were received as weanlings, aged for one month, and monitored for an additional two to seven months to support the following areas of experimentation: 1) the application of drugs or surgery alone or in combination with immunotherapy in aged guinea pigs or rats exhibiting neoplasia; 2) the long term immunization of guinea pigs to determine the antigenicity of tumors and leukemia either occurring spontaneously or induced by chemicals or viruses; 3) in vivo efforts to monitor cellular suppression following drug therapy or

surgery; and 4) long term observation for the development of metastatic lesions following surgical removal of the primary tumor. In addition, weekly shipments of leukemic and normal bloods, serum, plasma, peritoneal exudates, lymphatic tissue, tumor cells, and other organs have been sent to various NCI investigators.

Significance to Biomedical Research and the Program of the Institute: Existing facilities and space for new facilities devoted to the holding of small laboratory animals at the NIH reservation are extremely limited, with the result that necessary *in vivo* experimentation are severely curtailed. This contract provides holding facilities for small laboratory animals with a back-up laboratory support service that enables intramural investigators to continue research of high priority to the goal of the VOP.

Proposed Course: The holding and monitoring of animals to support the above described studies and the shipment of biological specimens to NCI investigators will continue.

Date Contract Initiated: June 15, 1971

Current Annual Level: $188,662

FLOW LABORATORIES, INC. (NO1-CP-6-1000)

Title: Repository for Storage and Distribution of Reagents, Sera, and Tissue Specimens

Contractor's Project Director: Mr. Harry F. Adkins

Project Officers (NCI): Dr. Jack Gruber
Dr. Lea I. Sekely

Objectives: This contract provides a low temperature storage facility for specially developed biological reagents and clinical specimens. The facility receives, inventories, and stores for Program use, research reagents, and normal and neoplastic human specimens, for further distribution to designated scientific investigators as authorized by OPR&L, NCI. Accurate inventories and records of distribution are provided to NCI. Additionally, the contractor applies adequate safety measures to maintain the integrity of all materials stored and shipped.

Major Findings: During the current period, the contractor made 975 shipments of viruses, viral reagents, and sera which comprised a total of 32,518 vials of material. The facility received 420 shipments of similar material which comprised 32,640 vials. All incoming shipments were carefully checked for damage in transit and were cataloged before being placed in the low temperature repository. During the same period, 3,630 tissue specimens were received. All tissue specimens were examined by a pathologist, classified as to tumor and tissue type, and cataloged and stored for further distribution. During this period 1,875 of these specimens were distributed to

17 investigators as authorized by NCI. In addition, 11,197 aliquots of sera from 1,605 donors were processed and added to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible, adequately characterized resource materials. The laboratory, storage, and shipping facilities operated under this contract enable collaborating investigators to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the activities of this contract will continue to provide rapid and flexible support to changing needs of the Viral Oncology Program and its collaborating investigators.

Date Contract Initiated: June 22, 1965

Current Annual Level: $222,042

FLOW LABORATORIES, INC. (NO1-CP-6-1012)

Title: Phase Out of Virus Research and Production

Contractor's Project Directors: Dr. R. Gilden
Dr. S. Oroszlan

Project Officers (NCI): Dr. Berge Hampar
Dr. Jack Gruber

Objectives: To provide an orderly phase out of NCI Contracts NO1-CP-5-3528, NO1-CP-5-3529 and NO1-CP-5-3530.

Major Findings: During the phase out period key personnel transferred and vital equipment was transported to other locations. This contract was in effect for only a few months and terminated on July 31, 1976.

Significance to Biomedical Research and the Program of the Institute: Facilitated orderly termination of relevant activities.

Proposed Course: This contract terminated July 31, 1976.

Date Contract Initiated: February 1, 1976

Current Annual Level: $3,004,368

HEALTH RESEARCH, INC. (RPMI) (NO1-CP-4-3392)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Directors: Dr. John W. Pickren
Dr. Joseph Sokal

Project Officers (NCI): Dr. Lea I. Sekely
Dr. David McB. Howell

Objectives: To collect tissues and blood samples from adults with malignancies for use by cancer researchers within the Viral Oncology Program.

Major Findings: Health Research, Inc. is located at the Roswell Park Memorial Institute in Buffalo, New York, one of the major centers for treatment of cancer in this country. During the past year 237 specimens from autopsy were shipped to the NCI repository at Flow Laboratories, Inc. The most common tumors were carcinoma of the ovary, carcinoma of the lung, carcinoma of the breast, malignant melanoma, malignant lymphoma and leukemia. Most of the tissue represented metastases involving liver, lung, lymph node, or bone marrow. Uncommon tumors were rhabdomyosarcoma and malignant teratoma. Moreover, 210 surgical and blood specimens were shipped to the NCI resources processing laboratory for rapid distribution. Thirty different types of tumors were represented with the most common being adenocarcinoma of breast, carcinoma of lung, sarcoma, carcinoma of ovary, carcinoma of endometrium, and Hodgkin's disease. Tissues were generally primary cancers and included neoplasms of breast, lymph nodes and lungs, livers, and ovaries. Spleen material from a hairy cell leukemia, bone from an osteogenic sarcoma, spleen material from an erythroleukemia and various tissue from rhabdosarcoma, melanoma and teratoma represented unusual types of cancer. The surgical and blood specimens shipped were from by-product materials remaining from standard surgical and clinical procedures after the necessary portions for diagnostic purposes were obtained.

Significance to Biomedical Research and the Program of the Institute: A major goal of the VOP is to isolate, propagate, characterize, and identify candidate human cancer viruses. Of paramount importance in the efforts to reach this goal is the continued availability of clinical specimens from patients with cancer. This institution has available a large number of cancer patients and, on a continuing annual basis, is in an excellent position to help meet increasing program needs for large quantities of human neoplastic specimens.

Proposed Course: It is anticipated that the increased rate of specimen acquisitions achieved during the past year will be continued.

Date Contract Initiated: June 22, 1972

Current Annual Level: $40,897

HEKTOEN INSTITUTE FOR MEDICAL RESEARCH (NO1-CP-4-3344)

Title: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Paul Szanto

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The objective of this contract is to provide sterile, viable, and freshly frozen normal or neoplastic human specimens to collaborating laboratories for biological, chemical, and virological cancer research investigations.

Major Findings: The Division of Pathology of the Hektoen Institute for Medical Research is affiliated with the Cook County Hospital in Chicago, Illinois. Last year this hospital admitted approximately 50,000 patients and the Pathology Division received over 20,000 specimens for diagnosis; in addition, there were autopsies performed. The principal investigator, the Director of the Pathology Division, made available a large number of human specimens not needed for clinical or pathologic diagnosis to investigators collaborating with NCI. A total of 485 sterile tissue specimens were shipped to the NCI resources processing center. Diagnoses represented carcinoma of the breast, ovary, stomach, kidney, esophagus; malignant lymphoma and Hodgkin's disease; liposarcoma, and a number of benign neoplasms and normal tissues. A total of 1,180 tumor and organ specimens were also shipped to the NCI repository. The tumors represented included carcinoma of the bladder, carcinoma of the cervix, lymphomas, leiomyosarcoma, angioma, and carcinoma of the pancreas. Normal adult and fetal tissues, and benign tumors were also supplied.

Significance to Biomedical Research and the Program of the Institute: A continuous supply of appropriate human tissues from patients with cancer is vital to cooperative cancer research investigations. In order to carry out important studies on the biochemistry and immunology of suspected oncogenic human viruses, it is imperative that large quantities of human malignant tissues be available for analysis.

Proposed Course: This contractor will continue to supply tissue specimens from patients with neoplastic disease and from normal individuals.

Date Contract Initiated: June 17, 1974

Current Annual Level: $139,330

HOSPITAL FOR SICK CHILDREN (NO1-CP-6-1040)

Title: Normal and Leukemic Human Tissue Collection

Contractor's Project Director: Dr. Peter D. McClure

Project Officers (NCI): Dr. Lea I. Sekely
Dr. Jack Gruber

Objectives: To gather serum, whole blood, and tumor specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls, during the course of normal clinical procedures.

Major Findings: During the year the contractor collected serum samples from new patients with leukemia and from patients on long-term follow-up for use by Virus Cancer Program collaborating investigators. Thirty-six tissue samples and 46 frozen and six fresh buffy coats from leukemia were shipped to the NCI resources processing center for distribution, while sera from 28 new leukemias, 77 follow-up leukemias, 57 new tumor cases, 47 follow-up tumor cases, 34 normal controls, and 12 hematologic controls, as well as nine whole blood samples, were shipped to the NCI repository. Attempts were made to isolate leukemic white cells in quantities greater than 1 gram, and deliver fresh specimens to NCI investigators within a 24 hour period.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to the VOP not readily available elsewhere. The contractor also provides an important service to Program by providing numerous samples of fresh and frozen buffy coats and tissue specimens.

Proposed Course: The contractor will continue to collect pediatric solid tumor tissue, sera, and leukemic white cells.

Date Contract Initiated: February 3, 1965

Current Annual Level: $25,000

HUNTINGDON RESEARCH CENTER (NO1-CP-3-3223)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers (NCI): Dr. David McB. Howell
Dr. Garrett V. Keefer

Objectives: The objectives of this contract are to develop, produce, and characterize special diagnostic reagents for use in the Viral Oncology Program. The special reagents consist primarily of antisera and

antisera conjugates to viruses, viral polypeptides, globulins of various animal species, and the T-antigen of SV40.

Major Findings: During this reporting period the contractor produced and characterized antisera against a very wide array of antigens encountered in cancer research, and added a number of antisera to his inventory. Among the new antisera added during this period are: anti-Gross virus p30; anti-FeLV p10; anti-FeLV p15; anti-FeLV glycoprotein; and anti-Balb-Virus 2 glycoprotein. During this report period the contractor distributed over 37,500 ml. of antisera to over 221 collaborating investigators in the VCP.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed and produced by the contract are vital tools in cancer research. The project functions in close collaboration with VOP research programs and is of very significant usefulness to the needs of the research community.

Proposed Course: To continue development, characterization, and production of antisera and serological test systems.

Date Contract Initiated: June 2, 1963

Current Annual Level: $359,625

ILLINOIS, UNIVERSITY OF (NO1-CP-4-3345)

Title: Supply of Human Materials Obtained from Patients with Neoplastic Diseases

Contractor's Project Director: Dr. Tapas K. Das Gupta

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The purpose of this project is to collect clinical by-product specimens of malignant human tumors and sera, pleural and ascites fluids, and whole blood from cancer patients for laboratory cancer research.

Major Findings: Specimens from a large number of cancer patients were obtained by the principal investigator from the University of Illinois Hospital, and VA West Side Hospital, and the Cook County Hospital in Chicago, Illinois. During the report period 106 solid tumors, 107 whole bloods, 23 sera, and 12 pleural and ascites fluids were shipped to the NCI resources processing center for subsequent rapid distribution to laboratory investigators.

Significance to Biomedical Research and the Program of the Institute: This project provided cooperating investigators with freshly-obtained specimens from cancer patients permitting them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: This contract terminated on September 30, 1977. The specimens provided through this contract are presently being obtained through other human resource contracts within the VOP.

Date Contract Initiated: June 17, 1974

Current Annual Level: $14,562

JEWISH HOSPITAL AND MEDICAL CENTER (NO1-CP-4-3251)

Title: Supply of Human Specimen Material from Patients with Malignancies

Contractor's Project Director: Dr. Harvey Dosik

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: To provide tissues and serum specimens from patients with various types of malignancies and genetic disorders related to cancer, as well as from patients with disorders of a non-malignant nature, to collaborating investigators for cancer research.

Major Findings: The Hematology Service of the Jewish Hospital, of which the principal investigator is Director, is the largest in Brooklyn with approximately 1,100 new in- and outpatient referrals per year. This large population of patients permitted the contractor during this report period to provide 699 serum samples from patients to the Program. In addition, 1,433 sterile and unsterile tumor specimens were collected from biopsy and autopsy procedures and shipped to the NCI resources processing center and the NCI repository. Moreover, 14 whole blood and buffy coat specimens were made available to NCI collaborating investigators.

Significance to Biomedical Research and the Program of the Institute: Fresh and frozen tissue and sera from patients with malignant disorders, related disorders, and individuals without malignancies are necessary for experiments to determine the etiology or etiologies of various malignancies. This contract effort is unique in that it has access to clinical by-product material from patients with chromosomal abnormalities who are known to be individuals with a high risk of cancer.

Proposed Course: Continue to supply normal and neoplastic tissues from individuals with chromosome abnormalities and various malignancies.

Date Contract Initiated: March 1, 1973

Current Annual Level: $65,000

JOHNS HOPKINS UNIVERSITY (NO1-CP-3-3245)

Title: Pediatric Tumor Resource

Contractor's Project Director: Dr. Herbert Kaizer

Project Officers (NCI): Dr. Paul Peebles
Dr. Jack Gruber

Objectives: The goal of this contract is the collection and distribution of biological materials related to pediatric malignant diseases.

Major Findings: This contract effort gathers clinical by-product material from an active pediatric oncology program at the Johns Hopkins Hospital. Its geographic proximity to the National Cancer Institute makes the effort an ideal resource of tumor material for a variety of studies being undertaken by the NCI Viral Oncology Program.

For the period of this report a total of 74 specimens from 47 different patients were transmitted. In general those materials included sera from patients and family members, body fluids containing tumor cells and solid tissues that became available from biopsy or necropsy, including tumor and uninvolved organs or adjacent tissues. Most solid specimens have been in excess of one gram and all materials, except those from necropsy, were transmitted sterilely within a few hours. The contractor also started a collection of sera obtained from oncology clinic patients and their families during the course of clinical activities. These sera have been transferred periodically in large batches to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: Detection, treatment, and prevention of human cancer requires an adequate determination of its etiology. Viruses have been implicated as possible causative agents in human cancer. Unfortunately, a lack of sufficient tumor material from patients in the pediatric age group limits investigations for oncogenic viruses that may have been vertically transmitted from mother to child. Materials from this contract will help make possible these imperative human studies.

Proposed Course: It is anticipated that this contractor will continue to supply unique pediatric specimens to NCI.

Date Contract Initiated: March 1, 1973

Current Annual Level: $27,000

LIFE SCIENCES, INC. (NO1-CP-3-3291)

Title: Production of Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Lea I. Sekely

Objectives: The objectives of this project are the large-scale in vivo production of BAI strain A avian myeloblastosis virus (AMV) and the preparation and distribution of significant quantities of AMV reverse transcriptase enzyme.

Major Findings: During the current period, the contractor obtained a total of 870 grams of AMV from the plasma of 363 chicks. Of this total amount of virus, over 190 grams were shipped as frozen plasma, 163 grams were shipped as fresh plasma or virus pelleted by centrifugation, 73 grams were used as an inoculum to produce more AMV, and 440 grams were used to prepare reverse transcriptase enzyme (RDDP).

Sixty-four investigators were recipients of plasma containing a total of 361 grams of AMV during this period; thirteen investigators received approximately 10,000 ml. of myeloblast cells of myeloblastic chicks; and over three million units of RDDP were sent to 226 scientists. Recipients of these materials were in laboratories throughout the world.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the VCP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced in vivo under controlled conditions which permit the study of the immunology, virology, biochemistry, and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man. Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: It is anticipated that the contractor will continue to meet requests for avian myeloblastosis virus and will continue the preparation of significant quantities of AMV reverse transcriptase enzyme.

Date Contract Initiated: April 19, 1971

Current Annual Level: $532,740

LIFE SCIENCES, INC. (NO1-CP-6-1005)

Title: Production of Germfree and Reagent Grade Specific Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Dr. Garrett Keefer
Dr. David McB. Howell

Objectives: To produce both germfree and specific-pathogen-free (SPF) animals for cancer research. SFP animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: During the report period one cubicle and one outbred expansion colony of isolator-derived BALB/c mice contained approximately 525 female breeders that supplied 270 timed-pregnant, 2,200 adult, and 625 weanling mice to user laboratories. The outbred BALB/c colony was begun with a small, inbred BALB/c stock of eight pairs maintained in a Reyniers isolator. One cubicle and two expansion colonies of 475 female, cesarean-derived, outbred NIH/Swiss mice supplied 536 timed-pregnant, 350 weanling and 445 young adult animals to user laboratories. An NIH Swiss nude mouse colony of 1,600 heterozygous female breeders, divided into four expansion colonies, supplied 225 time-bred, 2,940 homozygous weanlings, 531 heterozygous weanlings, 3,209 homozygous young adults, and 2,063 heterozygous young adults. A small nucleus of 125 NIH Swiss female mice are backcrossed with 8th generation NIH Swiss homozygous males to provide back-up reserves of heterozygous female breeders. A cesarean-derived nucleus of BALB/c nude, athymic mice was expanded to 20 male (homozygous) and 110 female (heterozygous) breeders. Expansion has been gradual due to a small starting nucleus and uppredictable inherent traits. Antibody testing of sera from BABL/c, NIH Swiss, and heterozygous females in the nude breeder colonies indicated all lines are free of six common murine viruses.

A total of 12,354 fertile quail eggs were supplied to user laboratories from a highly selected Japanese quail flock of 340 breeders maintained in a primary barrier cubicle. The isolator-derived flock was developed through elaborate trap nesting methods and recent monitoring in this laboratory indicates the line is free of common avian pathogens, including avian leukosis viruses and Marek's herpesvirus. An isolator-derived, pedigreed chicken flock of 215 female breeders supplied 5,203 fertile eggs and 2,260 nine-to-eleven day old embryos and 1,835 one-to-three day old chickens during the year. The flock was developed from 45 pedigreed chickens which consistently demonstrated high productivity over a period of several generations. The pedigreed flock was monitored at regular intervals during the laying cycle and following retirement at 11-12 months of age. Extensive monitoring indicates the flock contains no avian leukeosis virus, Marek's herpesvirus or other common avian pathogens. Regular phenotyping of eggs from pedigreed stock indicates the presence of C/O and C/B types. In subsequent generations, the phenotype may change from one type to mixed thus requiring

continual monitoring in each new flock to clarify status of each pedigreed bird.

Significance to Biomedical Research and the Program of the Institute: This contract provides VOP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of VOP activities as they occur.

Date Contract Initiated: February 8, 1968

Current Annual Level: $410,000

LITTON BIONETICS, INC. (NO1-CP-4-3260)

Title: Receipt and Distribution of Human Specimens

Contractor's Project Director: Mrs. Shirley Norris

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: This contract provided processing and logistics arrangements for the receipt and delivery of fresh human tissues for cancer research scientists.

Major Findings: Biological by-product materials handled by this project were available from routine clinical diagnostic and treatment procedures. These included tumor tissues, blood samples and other surgical and autopsy tissues contributed from 12 NCI resource contractors in the United States and Canada. During the final months of this effort, 463 specimens were received, processed and distributed to 27 investigators and the NCI repository. These included 376 solid tissues, 79 whole blood and bone marrow specimens and eight effusions. Forty-three donor collections of whole blood were processed for plasma. One placenta from a mother with a previous child with a malignant disease, obtained from the cooperating obstetrician, was divided and distributed to seven investigators and the NCI repository.

Significance to Biomedical Research and the Program of the Institute: This contract provided the processing, receipt, and distribution of fresh, viable human specimen material for cancer research purposes. The services

provided by this contractor are presently being performed by another contractor within the Program.

Proposed Course: This contract was terminated on October 31, 1976.

Date Contract Initiated: November 1, 1973

Current Annual Level: No funds from FY 1977

LITTON BIONETICS, INC. (NO1-CP-6-1006)

Title: Operation of a Facility to Provide and Maintain Subhuman Primates for Cancer Research

Contractor's Project Director: Dr. John Cicmanec

Project Officers (NCI): Dr. Garrett Keefer
Dr. Jack Gruber

Objectives: The objective of this contract is the maintenance of monkey breeding colonies and laboratories necessary for inoculation, care, and monitoring of primates.

Major Findings: During this report period efforts continued to provide both New World and Old World primates and biological materials from these animals for use by cancer research investigators, and to increase the size of the New World primate breeding colonies. The New World breeding colony now has 452 animals representing twelve species; 152 live births occurred in the breeding colony during this report period including 22 infants born in the cotton-topped marmoset (Saguinus oedipus) colony. The cotton-topped marmoset has been identified as an endangered species, which means they can no longer be imported into this country.

Approximately 284 macaques comprising three species were in the Old World breeding colony. A small breeding colony of gibbon apes (Hylobates lar) was also maintained. One hundred and four live births occurred in the Old World breeding colony, which included one birth in the gibbon ape breeding colony. Two hundred thirty-nine animals were assigned to 35 active special studies during this report period.

In support of these efforts, personnel in the Department of Pathology performed 145 complete necropsy procedures involving the preparation of 2,167 microslides (51 with special stains). The surgery team performed 28 major procedures to support the program, and the Department of Microbiology continued its support by performing specimen cultures, antibiotic sensitivity testing, and environmental monitoring. The Hematology Section performed 2,402 complete blood counts. Fifty-one coagulograms, 1,556 clinical chemistry procedures, and 7 bone marrow biopsies were processed. Routine monitoring

and cataloging of specimens continued in the Parasitology Section. A total of 43 tissue or serum samples were transferred or sent to outside investigators.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human cancer viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes.

Proposed Course: This contract will continue the following activities: the maintenance of breeding colonies of macaques and the expansion and maintenance of breeding colonies of 12 different species of New World primates; production of 200 to 225 infants of various species of both Old World and New World primates for use in experimental studies; continuing efforts in the establishment of a breeding colony of gibbon apes; and the long-term holding and study of experimental animals inoculated by collaborating investigators. All of the systems needed for the production, hand rearing, isolation, and proper care of the primate species represented are included within this program.

Date Contract Initiated: February 12, 1962

Current Annual Level: $1,370,000

LITTON BIONETICS, INC. (NO1-CP-6-1039)

Title: Holding Facility for Small Laboratory Animals

Contractor's Project Director: Dr. Peter Golway

Project Officers (NCI): Dr. Garrett Keefer

Objectives: The objective of this contract is to provide space for the holding and experimental manipulation of small laboratory animals for the direct support of Viral Oncology Program intramural investigators.

Major Findings: During this report period the contractor has received, maintained, monitored and experimentally manipulated a total of 17,465 mice, 99 rats and 10 rabbits in support of three VOP intramural investigators. Ten thousand experimental procedures have been performed, 9 transplantable mouse tumor lines have been established and a long-term mouse breeding colony for the development of mammary tumors has been started. More specifically, the experimental procedures included tumor protection tests, foot pad assays, the potency assay and safety testing of human skin test antigens, as well as the maintenance of 7 viral induced transplantable tumor lines.

Significance to Biomedical Research and the Program of the Institute: Existing facilities and space for new facilities devoted to the holding of small laboratory animals at the NIH reservation are extremely limited, with the result that necessary in vivo experimentation had been severely curtailed. This contract provides holding facilities for small laboratory animals with back-up laboratory support services that enable intramural investigators to continue research of high priority to the goals of the VOP.

Proposed Course: This support type contract that provides holding space for small laboratory animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet the changing needs of intramural investigators.

Date Contract Initiated: June 25, 1976

Current Annual Level: $237,000

MELOY LABORATORIES (NO1-CP-4-3263)

Title: Mouse Mammary Tumor Virus Production Facility

Contractor's Project Director: Dr. Bhalchandra Diwan

Project Officers (NCI): Dr. Wade Parks
Dr. David McB. Howell

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) to collaborating VOP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to produce MTV from tissue culture; and to develop improved methods for the propagation and detection of MTV and MTV antigens.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem. The current RIII mouse colony presently consists of approximately 2,300 breeding females, of which 1,700 are in the dairy facility. This number of animals is sufficient to produce approximately 275 ml. of milk per week.

A total of 17.6 liters of RIII milk was collected during the report period and 133 virus purification runs were completed yielding a total of 170 ml. of purified mammary tumor virus. A total of 60 mammary tumors have been recorded and distributed. In addition, the contractor has investigated methods for increasing the breeding efficiency of the animal production colony.

The following antisera have been prepared and distributed: rabbit anti-C_3H-MTV, guinea pig anti-RIII MTV, goat anti-MTV gp 52, and goat anti-gp 27.

MTV reagents have been distributed to over 41 investigators in the U.S.A. and also to a number of foreign laboratories.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. A major effort of the VOP is directed toward determining the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in humans may be developed.

Proposed Course: Purified MTV, viral reagents, and mouse milk will continue to be supplied as needed by VOP investigators.

Date Contract Initiated: January 13, 1974

Current Annual Level: $298,000

MELOY LABORATORIES (NO1-CP-5-3508)

Title: Support Services for Cell Biology and Cancer Research

Contractor's Project Directors: Dr. Kenneth Blackman
Dr. Michael Kelley

Project Officer (NCI): Dr. Charles W. Boone

Objectives: The purpose of this contract was to prepare virus-augmented human tumor skin test antigens, to provide radioimmunoassay capability, and to provide animal support facilities for NCI investigators.

Major Findings: This contract was terminated on August 19, 1976. During the month and one-half prior to termination, which is the time frame covered by this report, NCI personnel transferred government equipment and animals to other locations.

Significance to Biomedical Research and the Program of the Institute: Virus augmentation of human skin test antigens appeared to provide a sensitive immunological tool for early diagnosis and monitoring of the cancer patient. Resources and services furnished by this contract were used for further investigation of this phenomenon.

Proposed Course: This contract terminated on August 19, 1976, and essential activities are being continued elsewhere.

Date Contract Initiated: August 20, 1971

Current Annual Level: No funds from FY 1977

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-6-1038)

Title: Acquisition of Human Specimens for Use in Cancer Research

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officers (NCI): Dr. Lea I. Sekely
Dr. Garrett Keefer

Objectives: To collect sera and tissues from human subjects with neoplastic tumors to be used in cancer research studies by collaborating investigators within the Viral Oncology Program.

Major Findings: This contract represents one of the major acquisition and distribution efforts for the various human neoplastic specimen materials so necessary to the multifaceted research programs currently in progress that are seeking to ascertain the possible relationship of viruses to the etiology of human cancer. During the report period, the contractor provided to collaborating laboratories over 1,216 tissue specimens, including 779 bone marrow samples, and 203 specimens of white blood cells. Addditionally, 10,000 aliquots of sera were sent to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: Rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contractor will maintain the present high level of material supplied to the Program.

Date Contract Initiated: March 1, 1971

Current Annual Level: $190,515

MICHIGAN CANCER FOUNDATION (NO1-CP-4-3391)

Title: Collection of Large Quantities of Human Milk

Contractor's Project Director: Dr. S. Albert

Project Officers (NCI): Dr. E. Plata
Dr. David McB. Howell

Objectives: To collect large quantities of milk from women whose families have a history of breast cancer as well as from women whose families have no such history for use in cancer research investigations.

Major Findings: Human milk collected under this contract is sent to the NCI repository for general dissemination to collaborating scientists investigating the possible viral etiology of human mammary carcinoma. During the past year, the contractor interviewed 2,374 prospective nursing mothers, of which 52.8% were from non-cancer and 47.2% from cancer families. Nine hundred and thirty (930) milk kits were received from nursing mothers, of which 386 were from non-cancer and 544 were from cancer families. Approximately 88.4 liters of identified human milk were shipped to the NCI repository; of these, 30.9 liters were contributed by nursing mothers from non-cancer families and 57.5 liters by nursing mothers from cancer families.

Significance to Biomedical Research and the Program of the Institute: Particles with certain characteristics similar to animal oncogenic RNA viruses related to mammary carcinoma have consistently been detected in human milk; human milk is therefore currently a very important research material for studies on the etiology of human breast and other cancers.

Proposed Course: It is anticipated that the critical need for the material provided by this contract may be satisfied by the end of the contract year.

Date Contract Initiated: June 27, 1974

Current Annual Level: No funds from FY 1977

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Operation of a Service Laboratory

Contractor's Project Director: Dr. John C. Parker

Project Officers: Dr. Garrett Keefer (NCI)
Dr. Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of rodent viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations; and to assist in the characterization of the gene-dependent expression of murine leukemia.

Major Findings: The contractor operates a murine virus serodiagnostic and viral diagnostic laboratory for NCI. During this report period the serodiagnostic laboratory received 6,064 sera from research investigators utilizing animals. A total of 38,487 tests were performed on these sera: 2,929 mouse sera (21,199 tests), 146 rat sera (1,467 tests), 27 hamster sera (225 tests), 193 guinea pig sera (1,017 tests), 102 miscellaneous sera (153 tests), 750 MAP test sera (8,394 tests), and 1,907 internal supportive tests sera (5,978 tests). During this report period, the virus diagnostic laboratory received tumor fragments, cell cultures, viral reagents and tissues from diseased rodents to test for possible contamination. Out of

99 specimens examined, 36 were found to contain one or more virus contaminants. Twenty-nine specimens were positive for lactic dehydrogenase virus, 10 for minute virus of mice, 17 for polyoma, 1 for K virus, 4 for mouse hepatitis, 5 for lymphocytic choriomeningitis (LCM), and 2 for Reo type 3. In addition to the MAP tests, 12 of 66 tumors and cell cultures screened for LCM virus contamination were positive. Direct isolation of viruses from tissues of diseased animals in cell cultures was frequently used particularly when Sendai virus, mouse hepatitis virus, LCM virus, or ectromelia virus were suspected. The immunofluorescence capability has been expanded and the contract now can test for virus or antibody to 12 murine viruses. During this report period 262 fluorescent antibody tests were performed.

Reagents for approximately 36 murine viruses and serotypes are available for diagnostic tests; in this connection 1,955 ml. of viral reagents and 160 ml. of specific antisera were produced during this report period.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems.

Proposed Course: To continue the serodiagnostic services outlined above and to improve the sensitivity of the tests. To apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $475,000

MINNESOTA, UNIVERSITY OF (NO1-CP-6-1033)

Title: Development of Training Courses in Microbiological Safety

Contractor's Project Director: Dr. Donald Vesley

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives: The objective of this contract is to prepare for, and present, training courses entitled "Principles of Biohazards and Injury Control in the Biomedical Laboratory" and "Biohazard Containment and Control for Recombinant DNA Molecules".

Major Findings: During this report period the contractor presented six short courses: three for researchers working in disciplines related to viral oncology and three for researchers working with recombinant DNA molecules. The courses for oncogenic virology-related disciplines were held at

Massachusetts General Hospital, Boston, Massachusetts in October 1976; National Institutes of Health, Bethesda, Maryland in December 1976; and Northwestern University Cancer Center, Chicago, Illinois in June 1977. Two of the courses for the researchers working with DNA recombinants were presented during September 1976, one at Stanford University, Palo Alto, California on the 8th and 9th; and the other at Cold Spring Harbor, Cold Spring Harbor, New York on the 21st and 22nd. The third short course was presented at the Given Institute of Pathobiology, Aspen, Colorado in June 1977. A fourth short course is scheduled for the Frederick Cancer Research Center in September 1977.

Both laboratory safety training courses continue to emphasize the importance of contamination control principles and practices in protecting the experiment, laboratory workers, and the public. The contractor prepares for each course by recruiting students through personal letters to appropriate scientific investigators, and by placing notices in scientific and professional journals. Presently the contractor is editing new material for inclusion in the two course manuals and revising laboratory demonstrations for the Recombinant DNA course.

Significance to Biomedical Research and the Program of the Institute: The training program provides a most effective mechanism for disseminating current biological safety information and environmental control methods and techniques. This program benefits the institute by increasing the general safety awareness of the laboratory participant and his associates.

Proposed Course: Five courses will be scheduled for the coming year with more emphasis placed on the potential hazards associated with the use of chemical carcinogens and radiation procedures in cancer research.

Date Contract Initiated: December 10, 1971

Current Annual Level: $87,048

NAVAL BIOSCIENCES LABORATORY (YO1-CP-4-0200)

Title: Aerosol Properties of Potentially Oncogenic Viruses

Contractor's Project Director: Mr. M. A. Chatigny

Project Officer (NCI): Dr. A. Hellman

Objectives: To define the potential dosage to laboratory personnel from aerosols of cells and viruses generated during standard laboratory procedures. In addition, to study aerosol characteristics and to define the mode of transmission. Furthermore, the organization evaluates the optimum procedures and reagents required for surface decontamination and physical protection from various biohazards. The contractor also provides a local source of expertise in support of the Office of Biohazard Safety (NCI) to west coast contractors of the VOP.

Major Findings: Hypochlorite, ethanol, and isopropanol had previously been shown to be effective disinfectants for FeLV, RLV, HVS, and HSV. More recently SDS was tested and demonstrated to be an efficient disinfectant at 0.2%, particularly when used as a surface disinfectant. In contrast, several commercially available disinfectants were not very satisfactory.

Studies directed toward airborne HeLa cells as a cause of cell culture cross-contamination and as a vehicle for viral spread have been directed at establishment of appropriate culture conditions for HeLa cells. Preliminary stability studies indicate that cells can be retained in a viable state for up to several hours and may therefore become a ready means of cross-contamination.

The contractor has been extensively involved in the evaluation of an NCI facility to determine the potential of aerosol contamination. Tests were conducted using fluorescent tracer material and fluorometer detectors. Results from these studies are being assessed and a report transmitted to NCI. Site visit assistance to the OBS are continuing to contractors of the VCP located in the western portion of the U.S. During this period eight visits were performed with followup visits accomplished where appropriate.

A series of tests on the evaluation of the potential of pipettes as generators of aerosols were conducted with micropipettes, automatic pipettors and bulb-type devices. The aerosol output was in proportion to the size and volume of the pipette used. However, it was established that the requirement for cotton plugs in pipettes can be eliminated when using pipetting devices, thereby substantially reducing labor and supply costs.

Testing of safety cabinets indicates that there are currently two very efficient units commercially available. These cabinets are highly capable of product and operator protection.

Significance to Biomedical Research and the Program of the Institute: An understanding of survival and transmission characteristics of viruses and cells can provide a very effective indicator of potentials for cross-contamination, thereby reducing research and operating costs. The availability of efficient containment systems will also reduce the potential of human exposure to biological and chemical carcinogens. Elimination of cotton plugged pipettes appreciably reduces the time and cost in preparing certain laboratory equipment prior to usage.

Proposed Course: To continue studies of specific problems in control of laboratory biohazards generated in work with oncogenic viruses, chemical carcinogens, and infected cell cultures. Site visits and assistance to VCP contractors will continue to be assessed to assure reliability of facilities and techniques. Where appropriate, chemical and physical disinfectant techniques will be further evaluated and refined.

Date Contract Initiated: March 1, 1971

Current Annual Level: $41,012

PFIZER, INC. (NO1-CP-3-3234)

Title: Large-Scale Tissue Culture Virus Production for Cancer Research

Contractor's Project Director: Dr. Sami Mayyasi

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide a service facility for the production of large volumes of selected oncogenic and suspected oncogenic viruses, cellular antigens, tissue culture cell lines, and specific antisera to various oncogenic viruses. Production of these materials is supported by appropriate laboratory groups whose activities include process improvement, product standardization, quality control testing, and applied developmental research.

Major Findings: The current annual rate of large-scale tissue culture production results in over 25,000 liters of virus harvest fluids and over 4,900 grams of cells being processed to fulfill the needs of VOP collaborating investigators. During the past 15 months these materials were distributed in over 800 shipments to approximately 170 laboratories throughout the world. The major viral products generated included: Mason-Pfizer monkey virus (MPMV), which accounted for approximately 32% of the contractor's output; baboon endogenous virus (BeV), 25%; woolly monkey sarcoma virus (SSV-1), 21%; RD-114 virus, 13%; and Epstein-Barr virus. Of all viruses produced, more than 50% were grown on human cells (i.e. NC-37 lymphoblastoid line, A204 rhabdomyosarcoma line). The production of EBV in the P3HR-1 and B95-8 cell lines account for only approximately 1% of the total production effort, but the manipulation and concentration of these agents requires a disproportionate amount of time and effort as compared to the RNA type C virus production, and EBV preparation is a significant activity. Additionally, in support of collaborative studies being conducted at the IARC, Lyon, France, slides of concentrated P3HR-1 cells and RAJI cells are being produced and supplied to IARC for EBV epidemiologic titrations.

The isolation and characterization of the major proteins of all oncornaviruses in production has been accomplished. Antisera prepared against these purified antigens in rabbits are available to collaborating investigators upon request from NCI. The ability to provide reasonable quantities of these purified viral proteins and homologous antisera is a meaningful addition to the production activities performed by the contractor.

Applied studies are underway with both BeV and MPMV with the view of increasing the production levels of these viruses with respect to increasing 70S RNA content. Studies are underway exploring the host range for these agents and to optimize 70S RNA yield. Both nonhuman and human-derived cell lines are being utilized and progress to date has been excellent.

The current operational procedures employed in this laboratory consist of preclarification, and then concentration by molecular filtration and isopycnic banding using sucrose gradients in ultracentrifuges. Most clarification of virus harvest fluids are processed by employing continuous flow rotors in a

J-21 centrifuge. This is an efficient system for the complete removal of cells and cellular debris and results in excellent purification, but it is a time-consuming operation. Studies are underway employing nucleopor cartridge filters as on-line equipment for better preclarification. The use of molecular filters provides an option to the use of KII centrifuge concentration but studies have indicated that the biochemical properties of some viruses are better retained when molecular filtration is not employed. These studies are still in progress.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has been an invaluable back-up to intramural and collaborating investigators involved in virus-cancer research. The staff and facilities have been consistently responsive to changing needs of the National Cancer Institute. They have provided support to a wide variety of collaborating investigators making possible studies on viruses in cancer that could not otherwise be conducted. Recent research developments to determine the association of viruses with human neoplasia have involved activities concerned with molecular biology, biochemistry and immunology of oncogenic viruses. These types of investigations may provide clues to the mechanism whereby viruses mediate the transformation process. Such knowledge could indicate methods by which neoplastic transformation can be averted or inhibited and provide appropriate control measures applicable to the human cancer situation. Studies of this type have created a substantial demand for large quantities of concentrated and purified oncogenic and suspected oncogenic viruses. This contractor has both the capability to help meet such needs and the flexibility to quickly accommodate shifts in Program requirements.

Proposed Course: The production of viruses and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961

Current Annual Level: $1,628,800

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP-7-1014)

Title: Marmoset Colony for Cancer Research

Contractor's Project Director: Dr. Lauren G. Wolfe

Project Officer (NCI): Dr. Garrett Keefer

Objectives: The aim of this contract is the development and maintenance of a marmoset breeding colony in order to ultimately have appropriate numbers of these animals available for experimental use, and to provide a marmoset facility that includes a support laboratory for the inoculation and monitoring of animals under study as well as an adequate animal containment holding area.

Major Findings: This is a new contract effort that was initiated on April 1, 1977. The present colony consists of a total of 556 marmosets

including 94 cotton-topped marmosets (Saguinus oedipus), 428 white-lipped marmosets (S. fuscicollis), and 34 common marmosets (Callithrix jacchus). Colony components include two nurseries, one for uninoculated newborns and another for newborns inoculated with oncogenic material; three breeding colonies, one for each species of marmoset; an area with conventional caging for uninoculated animals; and an area for conventional or containment caging of experimental animals.

Clinical laboratory support has been provided for the routine monitoring of the animals and for performing selected techniques designed to improve the reproductive efficiency of the breeding colonies. The clinical service support includes hematology, microbiology, cytology, urinalysis, clinical chemistry, surgery and pathology.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. The marmoset appears to be especially suitable for use as a comparative model system. To date, at least five and possibly six virus tumor models, including Epstein-Barr and Herpesvirus saimiri viruses, have been established in marmoset monkeys. In addition, because of its small size the marmoset is more economical to house yet it is large enough for routine surgical procedures and serological monitoring.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: April 1, 1977

Current Annual Level: $353,310

ST. JOSEPH'S HOSPITAL (NO1-CP-3-3393)

Title: Tissue Procurement from Patients with Malignancies

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Lea I. Sekely
Ms. Wilma L. Varrato

Objectives: To supply investigators within the Viral Oncology Program with fresh specimens of tissue from human malignancies obtained as a byproduct of necessary surgical procedures.

Major Findings: During the current period, the contractor shipped over 400 tumor specimens from 321 patients and 616 sera or plasma to the NCI resources processing center and the NCI repository. These materials were either

distributed immediately in the fresh state to collaborating investigators or stored at low temperature for later research use.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent clinical information on the cases from which they came is essential to research on the possible role of viruses in human neoplasia.

Proposed Course: The contractor will continue the activities described above.

Date Contract Initiated: June 24, 1969

Current Annual Level: $61,427

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-3-3340)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officers (NCI): Dr. Garrett Keefer
Dr. David McB. Howell

Objectives: To breed, maintain, and supply young and mature chimpanzees to investigators for research in the sub-human primate on the possible viral etiology of human cancer.

Major Findings: The existing chimpanzee colony numbers 30 animals including three breeding males, eight breeding females, one juvenile male, nine juvenile females, one infant, and an additional eight infants assigned to five experimental studies initiated by collaborating Viral Oncology Program investigators. No live births were recorded during the past year. One female chimpanzee had a stillbirth in November and another aborted in December, 1976.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is currently the only source of newborn chimpanzees for the VOP.

Proposed Course: The chimpanzee colony will be maintained, and newborn animals will continue to be supplied to investigators within the VOP.

Date Contract Initiated: April 25, 1969

Current Annual Level: $75,000

STANFORD UNIVERSITY (NO1-CP-4-3244)

Title: Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. James T. Duff

Objectives: To serve as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera, cell cultures, and clinical materials submitted by NCI researchers and VOP contractors. Upon request, identification of isolates was made as to species.

Major Findings: The contractor completed the support effort requested and the activity terminated on August 31, 1976.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility was a testing and monitoring service available to all VOP investigators. Many of the most important viral specimens, cell cultures, sera etc., used in the viral oncology program were sent to this facility for mycoplasma testing.

Proposed Course: This contract effort terminated on August 31, 1976.

Date Contract Initiated: June 19, 1969

Current Annual Level: No funds from FY 1977

STANFORD UNIVERSITY (NO1-CP-6-1026)

Title: Development of a Method for the Large-Scale Production of DNA Ligase and DNA Polymerase I from *E. coli*

Contractor's Project Director: Dr. I. R. Lehman
Dr. Arthur Kornberg

Project Officers (NCI): Dr. Edward Scolnick
Dr. Robert Gallo

Objectives: To develop a method for production on a large scale of DNA ligase and DNA polymerase I and to utilize this method to produce these enzymes in quantities from ten to one hundred times greater at any given scale than methods previously available.

Major Findings: The contractor has constructed a hybrid bacteriophage lambda DNA containing the gene for *E. coli* DNA ligase. The original vector used to construct the lambda phage was λgt-λB, a strain that had been modified to incorporate endonuclease Eco RI generated restriction fragments. In this vector, the inserted DNA replaces the two central Eco RI fragments of lambda which are not required for growth. The fragment containing the ligase gene

was derived from a mutant strain of E. coli carrying a ligase overproducing mutation (lop-11). The lop-11 mutation itself leads to a 5-fold overproduction of ligase and infection of E. coli with a λgt-lop 11 lig+ hybrid phage resulted in a 10-fold increase in DNA ligase activity over that in extracts of uninfected lig+ cells.

In an effort to further increase the yield of DNA ligase, the contractor has modified the phage lambda DNA molecule to produce a vector which, under the appropriate conditions, generates extremely high levels of DNA ligase. The new vector λgt 4-lop 11 lig+, can form a stable lysogen which upon heat induction produces a 100-fold increase in DNA ligase activity. The introduction of a phage mutation (S7) that prevents cell lysis results in an even greater increase (500-fold) in DNA ligase activity.

Significance to Biomedical Research and the Program of the Institute: DNA polymerase I and DNA ligase have been of great value in a variety of studies directly related to cancer. Among these studies is the preparation of probes to determine whether certain RNA and DNA viruses may be involved in human malignancy.

Proposed Course: To develop a method for the purification of DNA ligase, and to construct a similar lambda phage containing the DNA polymerase I (poly A) gene.

Date Contract Initiated: December 22, 1975

Current Annual Level: $58,000

TENNESSEE, UNIVERSITY OF (NO1-CP-6-1020)

Title: Supply of Fresh and Frozen Brain Tissues for Cancer Research

Contractor's Project Directors: Dr. E. M. Stadlan
Dr. James T. Robertson

Project Officers (NCI): Dr. Lea I. Sekely
Dr. Gerald R. Suskind

Objectives: To supply NCI with fresh, viable, and frozen human brain tissue and brain tumors for subsequent distribution to investigators for cancer research.

Major Findings: The contractor is located in Memphis, Tennessee, which is the center for neurosurgical effort in the surrounding five-state area. A large number of neurosurgical procedures are performed in the participating hospitals, and the principal investigator, a neuropathologist, together with the co-principal investigator, the Chairman of the Neurosurgery Department, have access to all by-product brain material resulting from normal and standard surgical procedures.

During the current period 238 malignant and benign brain tissue specimens were collected. A total of 179 specimens from 188 subjects were shipped to the NCI repository. A total of 59 sterile specimens from 46 subjects were shipped to the NCI resources processing center. Tissue bearing the following diagnosis were shipped: astrocytomas, gliosis, meningiomas, pituitary tumors, acoustic neuromas, encephalomalacia, gliomas, metastatic tumors, schwannomas. In addition, one specimen was from a subject with two tumors, an astrocytoma and a meningioma.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract of major importance to the NCI, since it is a primary source of diverse brain tumor specimens for NCI researchers. A continuing supply of such specimens is necessary for the pursuit of the viral etiology of human cancer.

Proposed Course: To continue to supply fresh tissues to the Viral Oncology Program.

Date Contract Initiated: January 19, 1976

Current Annual Level: $40,000

TULANE UNIVERSITY (NO1-CP-3-3396)

Title: Maintenance of a Colony of Nursery-Reared, Seronegative, HVS-free Squirrel Monkeys

Contractor's Project Director: Dr. S. R. S. Rangan

Project Officers (NCI): Dr. Garrett Keefer
Dr. David Howell

Objectives: The maintenance and monitoring of a colony of Herpesvirus saimiri (HVS)-free, seronegative squirrel monkeys in order to have appropriate numbers of these animals available for experimental use.

Major Findings: The colony of HVS-free, seronegative squirrel monkeys was developed over a three year period as part of a multifaceted research contract effort. In June 1976 the research portion of the multifaceted contract was terminated and the animals became a Program resource. Currently the colony consists of a total of 41 animals (12 males and 29 females) ranging in age from 1½ to 4 years of age. These animals are housed in a specially fabricated facility far removed from the other squirrel monkey colonies. Admittance to these facilities is restricted to authorized animal care attendants and professional staff required for the maintenance of the colony. Personnel entering the seronegative squirrel monkey colony must adhere to strict clothing requirements and at no time handle or care for squirrel monkeys in the other HVS-seropositive squirrel monkey colonies. Periodic serological and virological monitoring of the animals indicates that they are free of HVS infection.

Regular observations of the older animals suggest a progressive decline in their abnormal behavior patterns, and an increase in their normal sexual activities as they near sexual maturity.

Significance to Biomedical Research and the Program of the Institute: This colony of seronegative, HVS-free squirrel monkeys provides a unique opportunity to evaluate the age-dependent host factors in HVS oncogenesis in the natural host, as HVS is endogenous in squirrel monkeys with the majority of feral and colony-reared animals possessing antibodies to the virus. Because the HVS-squirrel monkey animal model may resemble the relationship of EBV and Herpes simplex virus in humans, information gleaned from studies of HVS in seronegative squirrel monkeys may possibly be applicable to the human situation.

Proposed Course: The seronegative squirrel monkey colony will be maintained, and a breeding effort will be initiated with the sexually mature animals in the colony.

Date Contract Initiated: June 29, 1976

Current Annual Level: $51,000

UNIVERSITY HOSPITALS OF CLEVELAND (NO1-CP-6-1007)

Title: Supply of Pediatric Specimen Material from Neoplastic Diseases

Contractor's Project Directors: Dr. Samuel Gross
Dr. Robert Izant

Project Officers (NCI): Dr. Lea I. Sekely
Dr. Paul Peebles

Objectives: To procure whole blood, bone marrow, and tissues from pediatric subjects with neoplasms for use by collaborative cancer research investigators.

Major Findings: The active organization for this contract within the University Hospitals of Cleveland is the Department of Pediatrics at Rainbow Babies and Childrens Hospital, where Dr. Gross is director of the hematology-oncology section and Dr. Izant is director of pediatric surgery. The contractor is a primary, secondary, and tertiary hospital consisting of 97 surgical and 123 medical beds. The principal investigators have access to all pediatric material; during the current period, a total of 95 specimens were shipped to NCI resources processing center and to the NCI repository.

Significance to Biomedical Research and the Program of the Institute: The difference in the spectrum of tumors seen in childhood along with some apparent differences in their natural history and response to therapy make pediatric neoplasms an especially interesting class of tumors to study from a virologic, biochemical, and immunologic point of view. The availability

of sera and tissue materials from such pediatric patients make this contract a valuable resource for the NCI Viral Oncology Program.

Proposed Course: It is anticipated that the contractor will continue to provide an increasing number of pediatric specimens for the NCI.

Date Contract Initiated: October 15, 1975

Current Annual Level: $34,973

UNIVERSITY LABORATORIES, INC. (NO1-CP-3-3222)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. David McB. Howell
Dr. Jack Gruber

Objectives: To supply a variety of oncogenic viruses and their antisera in volumes necessary to meet VOP research needs.

Major Findings: The contractor's production of Rous sarcoma virus (Prague strain) in tissue culture roller bottles has continued at a very high level. During this report period, approximately 3,511 liters of virus-containing tissue culture fluid were produced; this material was pelleted, resuspended, and issued to various collaborating laboratories at approximately 132X of its original concentration. Production lots were sent to investigators in the U.S., France, West Germany, and the U.S.S.R. Recipients included Drs. Baltimore, Bishop, Joklik, Zamecnik, Zhdanov, Rabotti, Green and others.

The FeLV production effort was terminated in October 1976. During this report period the contractor produced approximately 1,750 ml. of feline leukemia virus (Rickard) which had been concentrated 10X. The virus, which was produced in tissue culture, was provided to numerous investigators.

The contractor initiated the production of BK virus in October, 1977. During this report period a total of approximately 4,265 ml. of BK virus has been shipped to NCI investigators for developmental research studies. Collaborative efforts between NCI scientists and the contractor have resulted in an almost two-fold increase in the DNA yield from the cell lysate material.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera produced by this contractor has been extensively used by VOP researchers and is essential to the continuation of many important research projects presently being carried out in the Program.

Proposed Course: Production of needed strains of oncogenic viruses and their antisera will continue in volumes necessary to meet VOP needs.

Date Contract Initiated: June 4, 1962

Current Annual Level: $333,853

WALTER REED ARMY INSTITUTE OF RESEARCH (YO1-CP-6-0503)

Title: Collection of Biological Specimens in Southeast Asia

Contractor's Project Director: Dr. Joseph Marshall

Project Officer (NCI): Dr. George Todaro

Objectives: The object of this contract is to collect a variety of wild mice in Southeast Asia for use by intramural NCI research investigators.

Major Findings: During this report period the contractor shipped to NCI laboratories a total of 464 mice comprising six species of the genus Mus, which were collected in six provinces in Thailand. The contract personnel in Thailand were responsible for: the collection and identification of animals of the genus Mus; the holding and maintenance of wild mice until they could be shipped to the U.S.; and the arrangements for shipping live animals from Thailand, including the screening of all mice for overt disease and the processing of the permits necessary for the export of animals from Thailand and the import of animals into the United States.

Significance to Biomedical Research and the Program of the Institute: Recent studies have shown that the endogenous type-C viruses isolated from Mus caroli and M. cervicolor are closely related to oncogenic viruses isolated from certain primates; that these primate viruses in turn appear to be related to oncogenes detectable in human tumors and tissue; that such viruses are not found in any of the other known species or strains of mice found elsewhere either feral or in the laboratory; and that these new viruses have been shown to be highly infectious in vitro for cells from a variety of other species including human. Hopefully the continued study of these viruses and their natural host would immeasurably aid in determining the possible viral involvement of human cancer.

Proposed Course: This contract was terminated on December 31, 1976.

Date Contract Initiated: July 1, 1976

Current Annual Level: $59,566

b. LITTON BIONETICS, INC. (NO1-CO-25423), BETHESDA, MARYLAND

Title: Operation and Maintenance of the Frederick Cancer Research Center at Frederick, Maryland

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Viral Oncology Coordinator (NCI): Dr. Henry J. Hearn

Objectives:

A. To isolate, purify and characterize by immunochemical techniques specific viral and viral induced proteins of biological, immunological, serological and virological importance to the Viral Oncology Program; to produce specific antisera to these proteins.

B. To conduct immunobiological surveys with reagents obtained above in model animal species and, where applicable, in man for evidence of oncornavirus expression, using radioimmunoassay and other techniques; to isolate and utilize tumor specific or related products for immunoprevention experiments in model systems.

C. To study control of expression and natural history of type B viruses (mouse mammary tumor virus, Mason-Pfizer, other non-C retraviruses), relation of virus expression to disease, attempt to control disease in natural host; to relate information and utilize reagents obtained to understanding human breast cancer.

D. To study biochemical and cellular factors concerned with the regulation of and assays for type C virus expression; to develop special mutant cell lines and somatic hybrids to study control of virus synthesis; to isolate functional viral proteins.

E. To conduct molecular biology investigations with types B and C oncornaviruses, generating specific probes for molecular hybridization for studies on viral interrelationships, viral replication, and transcription of the viral genome in normal and transformed cells.

F. To provide necessary immunology and molecular biology support for an ongoing collaborative program with NCI personnel investigating interrelationships among type C virus activation and normal host physiological processes, as possible mechanisms by which viruses influence or can be influenced by host-tumor interactions. Results of this work appear elsewhere in the annual report (Dr. Alfred Hellman).

G. Conduct in vivo and in vitro studies with primate herpesviruses, investigating the disease process involved in tumor development at the cellular and molecular level, to analyze the behavior of tumor viruses, tumor and transformed cells, immunological activity, and other host factors for the role they play in tumorigenesis, including the application of such studies to man.

H. To conduct studies on the isolation of single stranded HSV DNA probes for determining whether specific regions of the HSV genome are present in HSV transformed cells; to determine the polypeptide pattern of HSV-1 and -2 nucleocapsids to identify and characterize individual specific viral antigens, for use in studies on transformed and tumor cells of experimental and natural origin.

I. To provide necessary support for collaborative studies with NCI investigators on the mechanism of cell transformation by herpesviruses and their possible involvement as co-carcinogens with other viruses or chemicals, and for studies on alterations that occur in transformed cells, focusing on cellular enzymes, processes that repress or derepress normal cell components, and changes in properties of cell membranes induced by virus infection. Results of this work appear elsewhere in the annual report (Drs. Berge Hampar and Masakazu Hatanaka).

J. To provide necessary professional and technical support for a collaborative program with NCI personnel to develop appropriate genetically defined animal model systems for studying the influence of endogenous virus expression on normal cell differentiation and on abnormal changes leading to malignant transformation, to quantitate and analyze translational products of type C isolates, and genetically map selected type C RNA viral genomes. Results of this work appear elsewhere in the annual report (Dr. John Stephenson).

K. To provide necessary professional and technical support for a collaborative program with NCI personnel in evaluating various vaccines and vaccination protocols for their efficacy in preventing the expression of spontaneous, and chemically- or X-ray-induced neoplasia in animal test systems showing low to high expression of endogenous viruses. Results of this work appear elsewhere in the annual report (Dr. Robert Huebner).

L. To perform necessary collaborative electron microscopic support with an NCI investigator to develop new applications for the electron beam in understanding the structure and function of virus-cell interactions. In-depth discussion of this work can be found elsewhere in the annual report (Dr. Victor Zeve).

M. To conduct a major resource effort and provide facilities for the large-scale production of viruses or viral components that are necessary for performing laboratory research in the VO/FCRC or associated outside VO programs; to engage in developmental research on oncogenic or suspected oncogenic viruses necessary to the VO program but for which no previously established protocols exist or for which protocol improvement is required; to develop suitable quality control support activities involving electron microscopy, tests for contamination, and other appropriate biochemical, serological and biological tests to insure maximal quality of resources.

N. To conduct a comprehensive safety and environmental control program for the Frederick Cancer Research Center and to perform applied and basic studies and literature surveys for risk assessment in support of the various FCRC operations.

O. To operate an animal farm for the breeding of laboratory animals to meet the needs of research programs at FCRC, and for shipment to other NCI operations as production permits.

P. To provide an Animal Health Diagnostic Section for monitoring the health and genetic quality research animals at the FCRC and NCI in order to ensure the validity of animal-related research and to preclude the entry of undesirable pathogenic microbes, latent murine viruses and parasites.

Major Findings:

Those areas of Contractor operations that were completely funded by Viral Oncology are the following:

Immunochemistry and Immunobiology Laboratory: Woolly monkey and gibbon ape viral p30s yielded essentially identical peptide maps and no differences in the NH_2-terminal 28 amino residues were detected. Allowing for insertion of seven residues, the NH_2-terminal region of these p30s most closely resembled MuLV p30 among the mammalian viruses compared. Development of a method for reversible labeling of amino groups using C^{14} imidate esters is being applied to sensitive analysis of tryptic peptides. Antibodies to a synthesized common region of p30 molecules were raised and immunoassays developed. The murine virus GAG precursor protein was isolated by DNA-cellulose chromatography. The envelope glycoproteins of Rauscher virus, gp70 and gp45, were found to be immunologically and structurally highly related, the principal difference being carbohydrate content. The immune virolysis technique was utilized to study localization of virion polypeptides and specificity of component antisera. Antisera to p15(E) and gp70 (gp45) were lytic for MuLV while anti-p10, p15, p30 were not. Anti-MTV gp52 was lytic for MTV while other component antisera were not. Reagents and test systems for purification of FOCMA were developed and initial results indicate a strong membrane association. The NH_2-terminal sequence of bovine leukemia virus p25 was determined to be pro-leu-ser-glu-gly. This differs from all known type C virus p30s which initiate pro-leu-arg. Equine infectious anemia virus was found to have properties similar to the group of viruses which have a reverse transcriptase preferentially active with Mg^{++}. No antigenic relationship with known retraviruses was found. Despite extensive attempts no type C p30 antigenic reactivity has been found in human tumor tissues nor has antibody to this protein been found in human sera.

Tumor cells were found to have characteristic surface features not found in normal culture as detected by scanning electron microscopy. New immunolatex spheres were developed which give highly specific reactions with appropriate viral tests systems, e.g., anti gp70 but not p30 stains the surface of budding virions. This methodology complements other procedures using ferritin and peroxidase labels. Ultrastructural studies of bovine leukemia virus, squirrel monkey retravirus- and equine infectious anemia virus were made. These particles are all distinguishable from type C viruses and have distinctive features. The scanning electron microscope was utilized to correlate virus production based on other parameters with cell surface budding. A laboratory for mycoplasma screening was made operational.

RNA Virus Laboratory: Tissue-culture-produced MMTV was found to be oncogenic in weanling BALB/c mice. Precipitating antibody to intact virus preceded tumor formation in inoculated mice. A serum-free medium was developed and utilized to study effects of hormones and other additives on MMTV expression. Preliminary evidence of infection of hamster cells with MMTV accompanied by ability to grow in soft agar has been obtained. Evidence for distinct initiation sites for MMTV GAG and ENV genes has been obtained. Precursors of the respective virion polypeptides coded by these two genes have been isolated. High and low producer cell lines have been isolated for studies of protein and RNA expression. Two new MMTV cell lines have been developed, one for GR and one for C3H nursed on C57BL/6 mice. The latter could represent an endogenous MMTV. CMl and humoral antibody cytotoxicity studies with MMTV infected cells as a target have been initiated. Antisera to gp52 were cytotoxic while anti gp36 and p27 sera were not. Specific antibody to MMTV virions was found in mouse strains, including wild mice, especially where transmission of exogenous MMTV is established. A radial gel diffusion technique for MMTV p27 was developed and used for studies of antigen localization. Thus far, component sera reveal only gp52 and gp36 at the surface of MMTV. cDNA was prepared from tissue culture derived MMTV. This probe could distinguish infected from normal cell DNA both by extent of reaction and thermal stability.

MPMV passaged in human cells was found to have biological and biochemical properties identical to virus grown in rhesus monkey cultures. The squirrel monkey retravirus, SMRV, was distinguished from all previously described retraviruses based on polypeptide pattern and antigenicity. A low molecular weight DNA binding protein has been isolated from several type C viruses. This protein, plO, is highly basic and contains mainly group-specific antingenicity. Several amino acid analogs have been found to induce type C virus in BALB/c cells. This induction is not simply based on inhibition of protein synthesis and thus affords one approach to identification of specific "repressors". Drug resistant and temperature sensitive human cell cultures have been developed for potential use in assays of transformation-mutation by viruses, chemicals, and mixtures of the two. Collaborative studies on isolation of the GAG precursor by DNA-cellulose chromatography have proven successful with MMTV, MuLV, and the p60 protein of MI MSV (FeLV).

The feline oncornaviruses, FeLV and RD114, were found to be present in multiple copies in host cell DNA based on kinetic analysis and fractionation of DNA based on reiteration frequency. Smaller numbers of genome equivalents were found in heterologous infected cells. Sub-clones of non-producer mouse cells transformed by KiSV were found to vary in DNA and RNA content after BrdU treatment. Cells activated to produce virus had twofold more virus-specific DNA and tenfold higher RNA than cells remaining non-producer. Long length cDNA's of RLV, rat, and SSV viruses have been prepared for hybridization and planned transfection studies. Hydroxyurea was found to inhibit synthesis of (+) strand DNA in MuLV infected cells. Thirty minutes after removal of the drug, full length double-stranded DNA was found in the cell nucleus. Levels of cAMP effect xenotropic virus induction in an inverse manner. Thus far, baboon virus sequences (M-7) have not been detected in human cell DNA using probes which give 73-89% hybridization to baboon cellular DNA.

DNA Virus Laboratory: Herpes simplex virus inactivated by UV-light activated xenotropic MuLV in BALB/c cells. Induction was rapid, transient, and dose-dependent. HSV-1 and HSV-2 DNA have been prepared from media concentrates after labeling using KI isopycnic gradients. Mg active quantities of DNA, which is infectious and represents ~0.4 contour length, have been prepared. Methods for high-specific activity labeling are being evaluated. Using a gradient gel technique, HSV-1 and -2 nucleocapsids were found to contain six major polypeptides, of which, VP37 in HSV-1, and VP39 in HSV-2 appear to be internal proteins. Antisera to VP154, the major capsid protein, gave a unique nuclear fluorescence in infected cells. A virus-specific polypeptide, VP67, was found associated with the chromatin fraction of infected cells.

The immune response of owl monkeys with HVS induced lymphoma was studied in detail. Titers in the ADLC assay reached 1:60,000 or greater and correlated with loss of lymphocyte sensitivity to PHA. Responses of normal monkeys to T-dependent and independent antigens is under study. A factor produced by HVS lymphoid cells which blocks PHA responses is being characterized. Fibrinolytic activity was studied in lymphoid cells from several primate species, most showing activity; B-cells appeared most active. Lymphoid cell lines were established from cotton-topped marmosets with EBV-induced tumors. These were studied for surface markers, karyotype, and viral markers. Three strains of EBV were compared in vivo in marmosets and owl monkeys, each gave a distinctive pattern in antibody induction to the various viral antigens. A transforming baboon EBV-like virus was isolated from the colony at Sukhumi, USSR. This virus has not revealed an EBNA reactivity but shares capsid, early, and membrane antigens with EB. Foamy and cytomegaloviruses have also been isolated from the colony. An EB-like virus has been isolated from the peripheral blood of a leukemic orangutan. This virus induces an EBNA reaction as detected by both human and chimpanzee serum. Tumor cell cultures from a rhesus monkey and a galago have been initiated.

Disease Control Laboratory: Significant protection of AKR mice and their hybrids with low incidence strains against spontaneous leukemia was achieved with type C viral vaccines or passively administered anti-viral antibody. Protection was correlated with a decrease or elimination of ecotropic virus. Preliminary experiments indicate the possibility that anti-viral antibody will inhibit tumor induction in mice by chemical carcinogens. Tumors in rats were found to contain cross-reacting antigens detectable in complement fixation tests. Cross-protection in vivo was also demonstrated between two rat tumors of distinct origin. A transplantable, chemically-induced tumor was established in quail. Attempts to detect specific immune responses to this tumor are in progress.

Specific RIAs were established for avian GAG gene products for studies of precursors and restriction mechanisms. Genetic crosses in mice are in progress to correlate disease with expression of various classes of endogenous virus. Antigen expression of FeLV and RD114 viruses in cats is under study. An RIA for bovine leukemia p24 was developed and used to demonstrate both antigen and antibody in experimental and natural conditions. Specific immunoassays of structural proteins of baboon type C virus have been used to study the natural distribution of viral proteins in primate species. Genetic mapping of mammalian type C viruses is underway using specific immunoassays to detect viral subunits in precursor molecules, transformed non-producer cells, and mutant viruses.

Viral Resources Laboratory: During the 52 week period ending in January 1977, VRL produced 27,818 liters of oncogenic virus material - an average of 535 L/wk or 100% of total commitments (which were 300 L/wk through March and 600 L/wk for the remainder of the year). Production in the first six months totaled 11,949 liters, whereas 15,869 liters were produced in the latter six months.

Analysis of liters produced per agent for the year shows that MMTV (8,449 liters, 30%) was the most produced, followed by RLV (7,717, 28%), SSV-1 (3,496 liters, 13%), M-7 (2,906 liters, 10%), and GLV (2,798 liters, 10%). In addition, 2,452 liters (9%) were produced from a total of 19 (AKR, Balb-2, BLV, EIAV, FeLV, ASI, GALV-4, GLV, GLV L.p., M-7, MMTV, MPMV, KiMSV, M. caroli, RaLV, RLV, SMRV, SSV-1, and WB 334) different agents in the course of developmental studies. VRL released 28,488 milliliters of virus concentrate to authorized investigators. At the direction of OPRL/NCI, 17,971 milliliters of concentrate were distributed to 79 authorized program and foreign investigators. At the direction of the Director of Viral Oncology, 11,517 milliliters were released to FCRC investigators. At year's end, 2,557 milliliters of the 35,038 milliliter concentrate produced during the year remained in the VLR Virus Repository.

Those areas of Contractor operations that were partially funded by Viral Oncology are the following:

Environmental Control: Testing and certifying of laminar flow biological safety cabinets on receipt and on an annual schedule were continued. During the report period, Safety personnel tested cabinets for pressure tightness,

filter retention, uniformity of airflow, light intensity, noise level, and drip-tray leakage. All cabinets involved in annual inspections were decontaminated with formaldehyde gas prior to inspection. Forty-five decontaminated Type I and 17 Type II cabinets were involved in annual inspections and 22 Type I and 14 Type II were inspected upon initial installation. In general, the filters in the cabinets have been leak-free and the desired airflow was obtained by adjusting the motor controllers. Where leaks have been found in previously tested cabinets, the problem has been in the adhesive that attaches the filter medium to the frames. The repairs have been made relatively easily with RTV sealant.

A different brand of Class II, Type I cabinet (Nu-Aire) was introduced during the period. Of the seven cabinets tested, none were found to have particulate leaks in the shell, filter frame, or filters. They achieved the desired uniformity and volume of airflow without using Scott Foam. It is judged to be equal to the equivalent cabinet manufactured by the Baker Company.

Environmental Control personnel packaged a total of 358 biological, chemical or radiological shipments during 1976. Of this total, 326 were domestic shipments and 32 were foreign shipments.

A wide variety of other services has been offered from commenting and recommendations on laboratory procedures, review of drawings, checking Class I, Class III and chemical fume hoods, advising on specific hoods, approving clothing requests, and investigations of accidents or laboratory-associated problems. At the time of hood inspections, the general laboratory conditions are observed for general compliance with safety regulations.

Recently, consultations with the individuals responsible for establishing areas for working on the Recombinant DNA Program and with persons anticipating such work have become a significant duty. The introduction of this potentially hazardous program in Building 550 will require the greatest amount of monitoring of all the anticipated biological programs.

VO supported approximately 35 percent of this effort.

Animal Breeding: This project produces inbred, hybrid and outbred laboratory animals of various species and strains which are free of spontaneous diseases and of known genetic background. These animals are produced in quantities to meet the needs of the FCRC and other NCI operations as directed by the NCI Project Officer.

From January to December 1976, a total of 481,557 rodents and rabbits were issued to using laboratories. The breakdown by species was: 388,698 mice, 51,578 rats, 41,038 guinea pigs and 243 rabbits. Of this number 27 percent were issued to FCRC and NCI scientists at the Center, 19 percent to Bethesda and 54 percent to NCI scientists at other locations. Twelve production colonies have been established for the stated purposes of FCRC and NCI investigators.

The guidelines for establishing animal issue priority are enumerated in the FCRC Animal Farm Operation Policy, approved by the FCRC Steering Committee in 1975. It lists in decreasing priority the order for issuance of animals from Project 12 to using laboratories. They are:

(1) FCRC (including on-site NCI scientists)

(2) NCI (intramural research programs)

(3) Other NCI programs not related to FCRC or NCI, geographically or logistically and therefore could be contracted elsewhere.

Priorities for issuance of animals are established by the NCI Project Officer and are reviewed weekly by NCI. The numbers of animals issued to Priority 1 increased 9 percent over calendar year 1975.

With the FCRC animal production stabilized at maximum levels for the allotted space and the impending reduction of functional space experienced during each renovation of additional buildings, any establishment of new strains will impact proportionally on the numbers of established strains available in Categories 2 and 3 of the operational policy. Presently, all animals produced are issued at 4-5 weeks of age; space is presently unavailable for additional aging of issue animals.

The quality of animals produced at FCRC continues to be very high, and to assure their quality of health, the animals are routinely monitored by FCRC's Animal Diagnostic Health Section. All barrier mice and rats are free of viruses, pathogenic bacteria, and endo- and ectoparasites since their establishment in the barrier facilities three years ago. In addition, the conventional colonies are free of all pathogenic bacteria and parasites. The health profile sampling plan was formulated by the FCRC Information System's biostatistician and approaches a 1 percent sampling of the breeding colonies each month.

VO supported approximately 20 percent of this work.

Animal Health Diagnostic Section: The Animal Health Diagnostic Section was established to monitor and assure the health and genetic quality of all research animals at the FCRC and NCI. This is necessary in order to ensure the validity of animal-related research and to preclude the entry of undesirable pathogenic microbes, latent murine viruses and parasites. These agents can adversely affect or nullify research efforts in a number of ways. The AHDS also develops, coordinates, and disseminates the health and genetic profiles of different strains and species of research animals purchased from various commercial sources to ensure that all animals are of uniformly high quality. In order to safeguard the Center's existing programs, all animals purchased from commercial vendors as well as those arriving from other Institutes are quarantined for about two weeks during which time they are subjected to microbiological, parasitological, virological, and/or pathological evaluations by the AHDS. Unsatisfactory animals are destroyed.

The AHDS provides all of the above research support to NCI investigators at the Bethesda campus through the coordination of Dr. A. E. New, Director of Laboratory Animal Sciences, NCI.

The AHDS routinely monitors the health status of resident experimental animals from all the programs at FCRC and NCI, and provides clinical services. The Section also performs the quality assurance program for animal feed and bedding which should be free of undesirable biological and chemical agents.

VO supported approximately 20 percent of this work.

Significance to Biomedical Research and the Program of the Institute: The introduction of an administratively restructured VO/FCRC Program two years ago was followed by implementation of the Program as operational laboratory activities throughout last year. This has resulted in subsequent scientific accomplishments that are now furnishing increasing amounts of pertinent new basic and applied information on problems concerning the elucidation of the relationships of viruses to neoplastic disease relative to cause, detection, prevention and control. Significant experimental results on possible mechanisms of host-virus interactions, on biological, biochemical, and serological characteristics of oncogenic viruses and their components, on vaccine control and on potential relationships between viruses and human cancer have been obtained. In addition, a number of high quality viral resources and laboratory animals have been made available not only within FCRC but also to the outside scientific community in support of work in virology, immunology, molecular biology, genetics, and electron microscopy. Also, work supported by Viral Oncology continued to provide specific information to FCRC and outside laboratories on the handling of hazardous biological and chemical materials. Finally, as in past years, high caliber laboratory facilities are available for special investigations and for visiting scientists from both this country and abroad.

Proposed Course: The fact that the Contractor's new Director of Viral Oncology (DVO) has now fully and effectively assumed his responsibilities as the LBI FCRC leader of the VO/FCRC Program, and that the restructured effort and modified objectives are now being implemented, augurs well for a substantial period of overall relative program stability. At the same time, however, incorporated in the workscope is sufficient opportunity for enhanced program flexibility that will enable the DVO to develop the VO/FCRC research effort to maximal advantage.

As predicted in last year's report, the appointment of the Contractor's DVO has had a major positive impact upon the caliber and productivity of the research and resource areas, the former in particular. Plans are in effect for the DVO to continue basic investigations studying the natural history of oncogenic viruses using immunological, virological and molecular biologic methodology. Utilizing specific characterized viral products, e.g., viral envelope proteins and viral induced cell surface antigens, attempts will be made to interrupt viral, chemical and spontaneous neoplasia in appropriate models. Products of

tumor cell responses will be completely characterized and assayed for biologic activity. More specifically it is planned that work will proceed along the following lines: (a) Immunochemistry, for the isolation, characterization and antiserum production of important viral or viral-associated proteins; (b) Immunobiology of RNA viruses, for the development of radioimmunoassays for natural history surveys in appropriate test systems; (c) Molecular Biology, to prepare specific transcripts of RNA and DNA oncogenic viruses for studies of virus interrelationships, relationship of viruses to host genomes, transcription of viral genomes under experimental conditions, and preparation and analysis of transcripts specific for certain viral function; (d) Viral and Cell Biology, to develop assays for test viruses, optimal cell lines for virus expression and production, mechanism of virus induction, localization of viral genomes to specific chromosomes using somatic cell hybridization; (e) Primate (including man) Herpesviruses, to study transformation by these viruses or their infectious DNAs, to compare biological activities among various strains, to analyze immunological factors that are conducive or deleterious to cell transformation and tumorigenesis, and (f) Virus, Reagent, and Cell Production to provide a large-scale central resource for working materials.

Finally, fruitful collaboration will continue with on-site NCI investigators engaged in ongoing studies on genetic and vaccine control of virus expression, the carcinogenic and/or co-carcinogenic role of herpesviruses in cell transformation and tumorigenesis, cellular enzymes involved in processes repressing or derepressing cell components, cell membrane property changes induced by virus infection, and the influence of host physiology on oncogenesis and cellular control.

Date Contract Initiated: June 26, 1972

Current Annual Level:

	July 76-Sep 76 (TQ)	Oct 76-Sep 77
VO/FCRC Program	$1,686,381	$8,294,610
ISA*	175,850	967,924
Totals	$1,862,231	$9,262,534

*This represents Viral Oncology's portion of Contract NO1-CO-25423 Interdepartmental Support Agreement.

3. Program Management

a. Cancer Research Emphasis Grants (CREG)

The Virus Cancer Program funded its first Cancer Research Emphasis Grants during FY 1976. Two announcements asking for proposals were published in the NIH Guide on June 1, 1975. The titles were "Replication of RNA Tumor Viruses" and "Genetics of RNA Tumor Viruses." Thirty-nine proposals were received in response to the announcements and reviewed by Study Sections in January, 1976. Of these proposals, 13 were disapproved and 3 were deferred for site visits or further information; the rest were approved. The Virus Cancer Program funded ten of the approved applications, in order of priority, during FY 1976. Two additional applications were deferred for funding in FY 1977. A total of approximately $.75 million was obligated during FY 1976 as a result of the first two announcements.

The following grants were funded during FY 1976:

CA19341 Haseltine, Sidney Farber Cancer Center
"Replication of RNA Tumor Viruses"

CA19497 Kaji, University of Pennsylvania
"Studies on the Replication of RNA Tumor Viruses"

CA19558 Duesberg, University of California, Berkeley
"Physical, Chemical, Genetic Analysis of Tumor Virus RNAs"

CA19714 Somers, Eastern Virginia Medical Authority
"Genetics of Murine Sarcoma Virus"

CA19723 Wong, University of Illinois
"Genetics of Helper-Independent Mouse Sarcoma Virus"

CA19725 Vogt, University of Southern California
"Genetics of RNA Tumor Viruses"

CA19729 Watson, University of Montana
"Mechanism of Viral RNA-Directed DNA Polymerization"

CA19996 Green, St. Louis University
"Replication of RNA Tumor Viruses"

CA20011 Faras, University of Minnesota
"Mechanism of Oncornavirus Proviral DNA Synthesis"

CA20012 Kang, University of Texas
"Infectious Reticuloendotheliosis DNA Provirus"

On April 1, 1976 two further CREG announcements from the Virus Cancer Program appeared in the NIH Guide. The titles of these were "Malignancy Induced by Small DNA Viruses (Adenoviruses or Papovaviruses)" and "Herpesvirus-Induced Malignancy." In response to the announcements, 44 proposals were received and were reviewed by Study Sections in February and March, 1977. Of these proposals, 10 were disapproved and 34 approved. It is anticipated that the Virus Cancer Program will fund twelve of the approved applications, in order of priority, this fiscal year. A total of approximately $1 million will be obligated in FY 1977 as a result of these announcements and deferred funding of two applications from the second CREG announcement. It is anticipated that the following grants will be funded during this fiscal year:

CA19873 Lilly, Albert Einstein School of Medicine
"Genetic Control of Resistance to Friend Virus Disease"

CA19931 Lilly, Albert Einstein School of Medicine
"Mechanism of the H-2 Effect on Viral Leukemogenesis"

CA21768 Kasamatsu, University of California
"Polypeptides and the Protein-DNA Complex in SV40 Virian"

CA21771 Courtney, Baylor College of Medicine
"Studies of Purified Herpes Simplex Virus Glycoproteins"

CA21772 Lang, Duke University
"Leukemia and Cytomegaloviruses: Reciprocal Effects"

CA21776 Spear, University of Chicago
"Herpesvirus Gene Expression in Transformed Cells"

CA21788 Levine, Princeton University
"Cell Surface Antigens of Viral Transformed Cells and Teratomas"

CA21797 Gurney, University of Utah
"Growth Control and Viral Gene Expression"

CA21799 St. Jeor, Pennsylvania State University
"Herpesvirus-Induced Malignancy"

CA21801 Cabral, Baylor College of Medicine
"Herpesvirus Antigens in Transformed Cells"

CA21807 Zimmer, University of Kentucky
"Early Gene Transcription in Adenovirus 2-Infected Cells"

CA21808 Folk, University of Michigan
"Analysis of the Polyoma Genome in Transformed Cells"

CA21824 Green, St. Louis University
"Transforming Proteins of Three Human Adenovirus Groups"

CA21896 Eckhart, Salk Institute
"Transcription in Polyoma-Transformed Cells"

PROGRAM MANAGEMENT

b. Contract Reports

HAZLETON LABORATORIES, INC. (NO1-CP-6-1024)

Title: Support Services to Maintain Studies on the Role of Viruses in Experimental Oncogenesis and Human Cancer

Contractor's Project Director: Dr. David Valerio

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: The objective of this contract is to provide services in support of research into the role of viruses in expermental oncogenesis and human cancer.

Major Findings: During the past year, this project provided essential services to support in-house research of the Molecular Biology Section, Laboratory of RNA Tumor Viruses. The contractor prepared tissue culture medium according to rigid standards for sterility and cell viability and provided freezer space for storage of culture medium. The project produced special viruses and cells in large quantities for NCI staff and established and propagated human tumor cell lines and other special lines in tissue culture as reagents for the Viral Oncology Program.

The contractor also provided standardized assays according to established protocols for the detection and quantitation of type C viruses in samples supplied on a routine basis by NCI staff. These tests included radioimmunoassays for type C viral polypeptides, the reverse transcriptase assay, RNA-DNA and DNA-DNA hybridization, and biologic assays including the focus forming assay for sarcoma viruses and the X-C plague assay for leukemia viruses. Electron microscopy was also available as a service on a limited basis.

Other services provided by the project in support of NCI in-house research included glassware facilities and care and holding of animals. The project provided facilities which strictly adhered to biohazard regulations of NCI. These included separate laboratories for work with different viruses, including those classified as moderate risk agents.

Significance to Biomedical Research and the Program of the Institute: This project provides essential functions for highly productive in-house research whose goals are to establish a viral etiology of human cancer and to develop methods by which to prevent this disease. The project also provides the Viral Oncology Program important resources including human tumor cell lines established in tissue culture, specialized viruses, and testing services for detection and identification of new virus isolates.

Proposed Course: The contractor has proven expertise in providing these services and has a very high probability of continuing these efforts at a superior level of performance.

Date Contract Initiated: September 1, 1972

Current Annual Level: $726,395

LITTON BIONETICS, INC. (NO1-CP-4-3249)

Title: Support Services for the Application of Animal Virus Model Systems to Human Neoplasia

Contractor's Project Director: Dr. P. Weislogel

Project Officer (NCI): Dr. Robert Bassin

Objectives: The purpose of this project is to provide facilities and staff to support laboratory research investigations by intramural NCI scientists.

Major Findings: During the past 12 months the contract has supported NCI research investigators by the propagation and maintenance of approximately 100 cell lines, each in a well-defined, stable condition as required for particular experimental uses. It has also performed in vitro infectivity assays for murine sarcoma and leukemia viruses, at a level of approximately 100-300 assay plates in the 12-month period. It has also carried out over 39,000 assays for DNA polymerases in virus and cell preparations, and numerous complement fixation and radioimmunoassay tests for virus-related antigens.

Other support has included the production, characterization, and storage of new cell lines; production and characterization of virus stocks; fractionation of cell extracts for studies on virus-associated enzymes; and production and characterization of immune sera and immunocompetent cells.

Significance to Biomedical Research and the Program of the Institute: An understanding of viral defectiveness and the role "helper" viruses play is of value in determining the occurrence and mechanism of viral oncogenesis by type C viruses in man. The identification of viruses or viral products in human tumor cells is of value in assessing the role of viruses in human cancers, and ultimately, in developing techniques of both diagnostic and therapeutic significance. This contract provides the support facilities necessary for the carrying out of this research.

Proposed Course: The contractor will continue to provide the following services: maintain cell cultures in an optimal state for virus production; carry out quantitative virus assays; recover virus-related products from the cells themselves; monitor cell cultures for virus production by a variety of specific biological assay techniques as well as by specialized enzyme assays.

Date Contract Initiated: June 27, 1969

Current Annual Level: $515,000

MELOY LABORATORIES (NO1-CP-4-3207)

Title: Support Services for Studies on Spontaneous and Virus Induced Neoplastic Transformation

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. George J. Todaro

Objectives: The objective of this contract is to provide facilities and support services for intramural studies on spontaneous and virus-induced neoplastic transformation.

Major Findings: During the past year this contract provided necessary facilities and services to support in-house research of elements of the Laobratory of Viral Carcinogenesis, VOP, DCCP, into the viral etiology of human cancer. Among the services furnished by the contractor were: a) preparation and formulation of tissue culture media; b) maintenance of human and non-human cell lines; c) large-scale production of cells; d) initiation of new cell lines; e) maintenance of animal facilities; f) maintenance of stocks of oncogenic and potentially oncogenic human and animal viruses; g) routine serologic and biochemical assays for viruses; h) routine nucleic acid hybridizations; i) immunization of animals; j) routine immunofluorescent assays; k) electron microscopy l) histology and pathology; and m) glassware services.

Significance to Biomedical Research and the Program of the Institute: Important findings regarding the mechanism of action of oncogneic viruses, their effects on cellular growth control mechanisms and their possible involvement in natural oncogenesis have been developed from a number of projects on this contract. A number of new type C viruses have been isolated and characterized and have provided much information on the nature of type C virus-cell interactions. Hopefully, the techniques employed in inducing and growing these viruses may be used to isolate a complete human type C virus which would immeasurably aid in studies of the possible viral involvement in human cancer. The services afforded by this contract are of essential importance in furthering this research.

Proposed Course: To continue to provide services and facilities in support of the indicated research.

Date Contract Initiated: May 25, 1965

Current Annual Level: $644,965

MELOY LABORATORIES (NO1-CP-4-3223)

Title: Support Services for Molecular Studies of Human and Animal Cancer with Emphasis on Mammary Carcinoma

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: The objective of this contract is to provide support services and facilities for molecular and immunologic studies of human and animal cancer with emphasis on mammary carcinoma.

Major Findings: During the past year, this contract provided necessary facilities and services to support in-house research of elements of the Laboratory of Viral Carcinogenesis, VOP, DCCP, concerning the viral etiology of cancer in experimental animals and humans. Among the services furnished by the contractor were: preparation and formulation of tissue culture media; maintenance of human and non-human cell lines; large scale production of cells; initiation of new cell lines; maintenance of animal facilities; maintenance of stocks of oncogenic and potentially oncogenic primate and rodent viruses; routine biochemical assays for viruses; routine nucleic acid hybridizations; immunization of animals; electron microscopy; glassware services; and preparation of viral structural proteins and enzymes used to study RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The ability to detect viral markers in human tissues is basic in the establishment of an etiologic relationship between viruses and cancer and may prove extremely useful in the early detection and treatment of certain forms of cancer. This contract provides services and facilities essential to studies whose prupose is to develop this ability.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: July 20, 1973

Current Annual Level: $739,177

MELOY LABORATORIES (NO1-CP-4-3236)

Title: Support Services for Immunological and Biochemical Studies of Mammalian Viral Oncology

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Edward Scolnick

Objectives: the purpose of this contract is to provide support services and facilities for studies on the immunology and biochemistry of mammalian viral oncology.

Major Findings: During the past year this contract provided essential services and facilities to support in-house research of the Laboratory of Tumor Virus Genetics, VOP, DCCP, concerning the viral etiology of human cancer. Among the services furnished by the contractor were: preparation and formulation of tissue culture media; maintenance of human and non-human cell lines; large-scale production of cells; initiation of new cell lines; maintenance of animal facilities; maintenance of stocks of human and animal oncornaviruses; routine biochemical assays for viruses; routine nucleic acid hybridizations; immunization of animals; routine immunofluorescent assays; electron microscopy; glassware services; and preparation of viral structural proteins and enzymes and cellular enzymes used for the study of RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The ability to detect viral information in transformed cells is basic to establishment of etiological association and to an ultimate approach to prevention or treatment of cancer. This contract provides services and facilities essential to studies whose purpose is to develop this ability.

Proposed Course: Continuation of support services as described.

Date Contract Initiated: May 25, 1965

Current Annual Level: $937,746

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-4-3254)

Title: Support Services to Maintain Studies of Type C RNA Tumor Viruses

Contractor's Project Director: Dr. P. Reddy

Project Officer (NCI): Dr. Padman Sarma

Objectives: To provide support services for type C RNA tumor virus studies carried out by NCI personnel or under NCI direction at the contract facility.

Major Findings: The scope of the work was limited to 17 specifically defined service functions itemized under two broadly divided tasks: (1) Virology, and (2) Immunology and Molecular Biology. Services provided under these tasks include isolation of type C viruses; preparation of tissue cultures (primary and established lines); infectivity and other assays for type C viruses; purification of viruses; storage and retrieval of cell cultures and viruses at ultra low temperatures; maintenance of cell repository; determination of infectivity and host range studies using various target cells; procurement and establishment of tumor tissues in culture as designated by the Project Officer; animal inoculation; maintenance of animal facility; collection and testing of sera for sero-epidemiologic survey; preparation of tissues and tissue culture antigens for immunologic procedures; production of high titer monospecific virus neutralizing antibodies; performance of immunologic tests such as immunodiffusion, complement fixation, and immunofluorescence for virus detection;

preparation of antigens and antibodies for radioimmunoassays; preparation of reagents for molecular hybridization with DNA and RNA probes of selected viruses and cellular DNA's; carry out reverse transcriptase assays for the detection of viruses using standard dT·rA template.

Significance to Biomedical Research and the Program of the Institute: The studies supported by the services of this contract provide further information on the natural occurrence and spread of oncogenic type C viruses in homologous and heterologous species, including man.

Proposed Course: Support services will be continued for demonstration, isolation, and characterization of endogenous type C viruses of rat, cat, turkey, nonhuman primates, and man, as listed above.

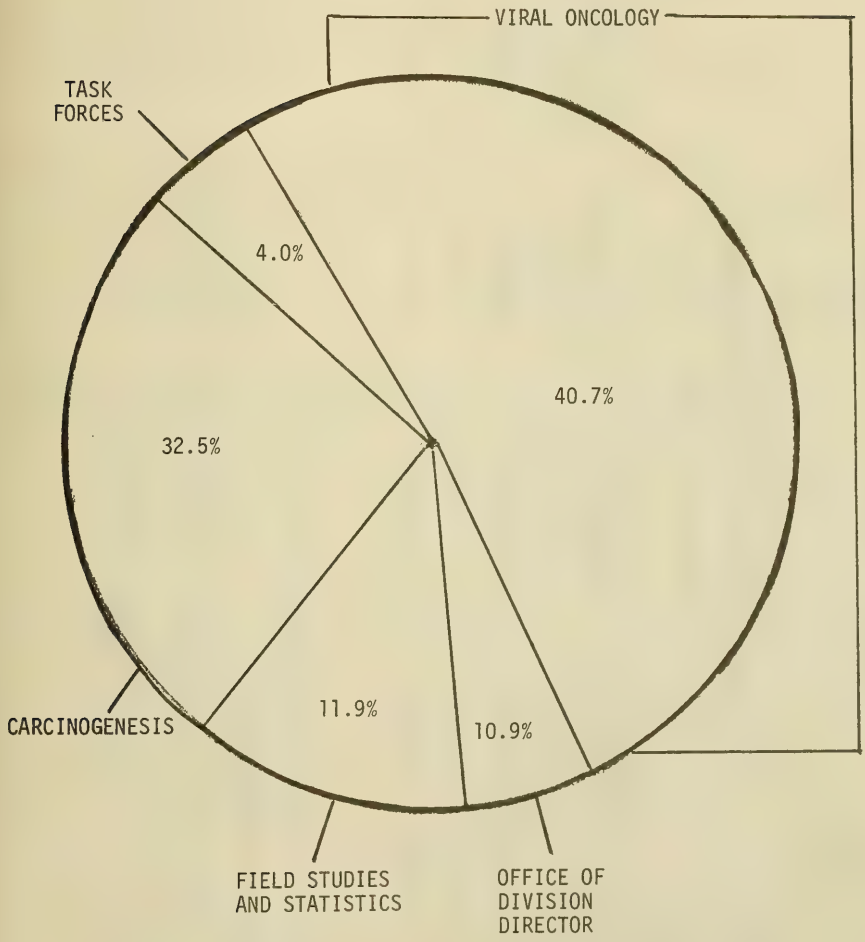
Date Contract Initiated: October 23, 1973

Current Annual Level: $359,862

NATIONAL CANCER INSTITUTE

Division of Cancer Cause and Prevention

Current Distribution of Funds - Fiscal Year 1977



DIVISION OF CANCER CAUSE AND PREVENTION

NCI

Table of Ratios of Contracts to
Intramural Effort Based on
Estimated Current Level of Expenditure**

September 30, 1977

	ODD	FIELD STUDIES AND STATISTICS	CARCINOGENESIS	VIRAL ONCOLOGY	TASK FORCES	TOTAL
Total	15,950,000	17,957,000	46,642,000	58,797,000	5,734,000	145,080,000
Contracts	13,235,000	11,557,000	39,619,000	43,200,000	5,085,000 1/	112,696,000
CREG	800,000	1,750,000	1,150,000	1,700,000	0	5,400,000
Intramural*	1,915,000	4,650,000	5,873,000	13,897,000	649,000 2/	26,984,000

---

* Includes cost of managing contract activities

** Based on FY 1977 Apportionment

1/ Includes $3,635,000 for Lung Cancer (Carcinogenesis Area); and $1,450,000 for Breast Cancer (Viral Oncology Area).

2/ Direct operations for Lung Cancer (Carcinogenesis Area)

Note: Construction not included.

OFFICE OF DIVISION DIRECTOR

Peters, James A.	Director
Gori, Gio B.	Deputy Director
Flamm, W. Gary	Assistant Director
Kraybill, Herman F.	Sci. Coord. for Environ. Cancer
Depue, Robert H., Jr.	Scientist Director
Owen, Thomas B.	Microbiologist
Sontag, James	Microbiologist
Cameron, Thomas	Veterinary Officer Director
Recer, James	Expert
Munn, John	Pharmacologist

Administrative Management Branch

Miller, John M.	Administrative Officer, Chief
Olimpio, Nicholas A.	Administrative Officer
Velthuis, Robert, Jr.	Administrative Officer, Viral Oncology
Crane, Damian	Administrative Officer, Carcinogenesis
Prather, James	Admin. Officer, Field Studies & Stat.

Office of Associate Director for Field Studies & Statistics

Schneiderman, Marvin A.	Associate Director
Fox, Bernard H.	Research Psychologist
Fears, Thomas R.	Staff Fellow
Land, Charles	Expert
Boyd, Suellyn	Staff Fellow

Biometry Branch

Office of the Chief

Vacant	Chief
Murray, James L.	Vet. Officer Director
Levin, David L.	Sr. Surgeon
Brown, Charles C.	Res. Math. Statistician
Locke, Frances B.	Statistician

Automatic Data Processing Management Section

Weiss, Theodore	Digital Comp. Systems Admin., Head
Larson, James E.	Computer Systems Analyst
Stump, James M.	Computer Systems Analyst
Cusano, Mary M.	Computer Specialist
Van Holten, Valerie	Computer Specialist
Gales, Altamease A.	Computer Programmer
Myer, Margaret D.	Computer Systems Analyst

Biometry Branch (continued)

Automatic Data Processing Management Section (continued)

Jenkins, George R.	Computer Programmer
Hollingsworth, V. Calvin	Computer Specialist
Shevchenko-Mason, Anya	Computer Specialist

Clinical & Diagnostic Trials Section

Byar, David P.	Medical Officer, Head
Gail, Mitchell H.	Sr. Surgeon
Corle, Donald K.	Computer Systems Analyst
Koziol, James A.	Staff Fellow
Muenz, Larry R.	Mathematical Statistician

Demography Section

Young, John L., Jr.	Sr. Scientist, Head
King, Haitung	Research Sociologist
Scotto, Joseph	Sr. Health Services Officer
Connelly, Roger R.	Statistician (Health)
Percy, Constance L.	Survey Statistician (Health)
Horm, John W.	Sr. Asst. Health Serv. Officer
Devesa, Susan S.	Statistician (Health)

End Results Section

Myers, Max H.	Mathematical Statistician, Head
Lourie, William I., Jr.	Statistician (Health)
Axtell, Lillian M.	Supv. Statistician (Health)
Hankey, Benjamin F.	Res. Math. Statistician
Prorok, Philip C.	Mathematical Statistician
Baranovsky, Anne	Statistician (Health)
Heise, Herman W.	Statistician (Health)
Shambaugh, Evelyn M.	Statistician (Health)
Asire, Ardyce J.	Statistician (Health)
Silverman, Debra T.	Statistician (Health)
Bundy, Brian	Mathematician
Gloeckler, Lynn	Statistician (Health)
White, Paulette	Mathematical Statistician

Field Liaison Section

Geller, Harvey	Statistician (Health), Head
Cunningham, Jack	Medical Record Librarian

Biometry Branch (continued)

Mathematical Statistics & Applied Mathematics Section

Gart, John J.	Res. Mathematician, Head
Connor, Robert J.	Res. Math. Statistician
Pettigrew, Hugh M.	Res. Mathematician
Thomas, Donald G.	Res. Math. Statistician
Layard, M. W.	Sr. Staff Fellow
Nam, Jun-Mo	Math. Statistician
Tarone, Robert E.	Staff Fellow
Smith, Alroy M.	Statistician (Health)

Environmental Epidemiology Branch

Office of the Chief

Fraumeni, Joseph F., Jr.	Chief, Medical Director
Blattner, William A.	Surgeon
Blot, William J.	Statistician (Health)
Burton, George	Scientist Director
Stone, Betty	Expert
Decoufle, Pierre	Statistician (Health)
Pickle, Linda	Staff Fellow
Yanagawa, Takoshi	Visiting Scientist
Brinton, Louis	Epidemiological Res. Asst.
Thomas, Terry	Sociologist

Environmental Studies Section

Hoover, Robert N.	Senior Surgeon, Head
Mason, Thomas J.	Statistician (Health)
Hayes, Howard	Veterinary Medical Officer
Blair, Aaron	Staff Fellow
Ramsbottom, Richard	Computer Specialist
Stephenson, Boyd	Computer Specialist
Dalager, Nancy	Computer Programmer
Jones, Nancy	Statistical Assistant

Clinical Epidemiology Branch

Office of the Chief

Miller, Robert W.	Medical Officer, Chief
Beebe, Gilbert	Expert
McKay, Frank	Computer Specialist

Clinical Genetics Section

Mulvihill, John J.	Suregon, Head
Satyer, Andrew	Geneticist

Epizoology Section

Priester, William A., Jr.	Veterinary Officer, Head

Special Cancer Studies Section

Miller, Robert W.	Acting Head

Clinical Studies Section

Li, Frederick P.	Sr. Surgeon, Head
Marchetto, David	Epidemiological Technician

Office of Associate Director, Carcinogenesis Program

Gori, Gio B.	Acting Associate Director
Cooper, John A., II	Chemist, Deputy Assoc. Director

Office of the Coordinator for Collaborative Research

Owen, Thomas B.	Acting Chief
Dunkel, Virginia C.	Microbiologist
Kaplan, Ann E.	Chemist
Smith, Carl E.	Chemist
Longfellow, David	Biologist (Sci. Adm.)

Carcinogen Bioassay and Program Resources Branch

Office of the Chief

Cueto, Cipriano	Pharmacologist, Acting Chief
Siegel, Sidney	Biologist
Chu, Kenneth C.	Chemist
Chase, Joan W.	Tech. Info. Spec. (Phy. Sci.)
Linhart, Mary S.	Computer Programmer
Orme, Thomas	Microbiologist
Douglas, Fielding	Expert

Carcinogen Metabolism and Toxicology Branch

Office of the Chief

Weisburger, Elizabeth K.	Scientist Director, Chief
Evarts, Ritva	Veterinary Medical Officer
Grantham, Preston	Research Chemist
Benjamin, Timothy	Research Chemist
Brown, Carolyn	Microbiologist
Elliott, Jerry	Microbiologist
Mohan, Letitia	Chemist

Nutrition and Metabolism Section

Poirier, Lionel A.	Supv. Research Chemist, Head
Yamamoto, Richard	Research Chemist
Wilson, Mary	Chemist

Analytical Chemistry Section

Keefer, Larry K.	Supv. Research Chemist, Head
Roller, Peter P.	Research Chemist
Miller, John R.	Chemist

Biology Branch

Office of the Chief

DiPaolo, Joseph A.	Research Pharmacologist, Chief

Somatic Cell Genetics Section

DiPaolo, Joseph A.	Head
Myhr, Brian	Sr. Staff Fellow
Popescu, Nicolae	Visiting Associate
Donovan, Paul	Chemist
Nelson, Richard	Biologist

Tumor Biology Section

Evans, Charles H., Jr.	Sr. Surgeon, Head

Chemistry Branch

Office of the Chief

Gelboin, Harry V.	Chief
Belvedere, Giorgio	Visiting Scientist

Cell Growth Regulation Section

Kakunaga, T.	Expert, Head
Hatfield, Dolph	Research Biologist
Caicuts, Mary Hane	Research Chemist
Kraemer, Kenneth	Senior Surgeon
Waters, Haywood	Biologist

Molecular Carcinogenesis Section

Gelboin, Harry V.	Acting Head
Okano, Paul	Staff Fellow
Deutsch, Joseph	Visiting Scientist
Leutz, Janet	Chemist
Robinson, Richard	Biologist

Nucleic Acids Section

Peacock, Andrew	Acting Head
Kakefuda, Tsuyoshi	Medical Officer, Research
Day, Rufus	Research Phys. Sci.
Bak, Tina	Microbiologist
Crow, Janet	Biologist

Protein Section

Peacock, Andrew	Research Chemist, Head
Green, Marie	Research Chemist
Bunting, Sylvia	Chemist
Pastewka, Jullia	Chemist
Reed, Roswell	Biologist

Experimental Pathology Branch

Office of the Chief

Saffiotti, Umberto	Chief
Levitt, Morton	Surgeon

Differentiation Control Section

DeLuca, Luigi	Research Chemist, Head
Jones, Carol	Microbiologist

Tumor Pathology Section

Goodman, Dawn	Vet. Med. Officer, Acting Head
Lingeman, Carolyn	Medical Officer, Pathology
Barrettt, Margaret	Biologist
Keller, Jean Marie	Biologist Lab. Tech. (Microbiology)

Human Tissue Studies Section

Harris, Curtis C.	Sr. Surgeon, Head
Hsu, Ih Chang	Expert
Stoner, Gary	Expert
Iodine, Jane	Research Chemist
Jackson, Frank	Biologist
Yamaguchi, Maria	Biological Lab. Tech. (Microbiology)

Perinatal Carcinogenesis Section

Rice, Jerry	Sr. Scientist, Head
Chen, Beatrice	Research Chemist
Berman, Jules	Staff Fellow
Ben, Theresa	Chemist
Dove, Lee Franklin	Bio. Lab. Tech. (Animal)
White, Wesley	Biologist

In Vitro Pathogenesis Section

Yuspa, Stuart H.	Senior Surgeon, Head
Hennings, Henry	Research Chemist
Morgan, David L.	Biologist
Colburn, Nancy	Expert
Lichti, F. Ulrike	Expert
Poirier, Miriam	Chemist

Lung Cancer Branch

Office of the Chief

Sporn, Michael B.	Medical Director, Chief
Frolik, Charles A.	Sr. Staff Fellow
Reese, David	Sr. Staff Fellow
Roberts, Anita	Research Chemist

Lung Cancer Branch (continued)

Office of the Chief (continued)

Smith, Joseph	Biologist
Friedman, Rosalind	Chemist
Lilga, Juliet	Microbiologist
Newton, Dianne	Research Chemist
Tavela, Thomas	Chemist

Lung Cancer Pathogenesis Section

Sporn, Michael	Acting Head

Office of the Associate Director, Viral Oncology Program

Moloney, J. B.	Associate Director
Sibal, Louis R.	Deputy Associate Director
Hearn, Henry J.	Microbiologist (FCRC)
Goldberg, Robert J.	Microbiologist
Reisinger, Robert C.	Veterinarian
Zeve, Victor H.	Research Biologist (FCRC)
Howell, David	Microbiologist
Ovuka, Margaret	Occupational Health Nurse

Office of Program Resources & Logistics

Gruber, Jack	Microbiologist, Chief
Keefer, Garrett V.	Microbiologist
Sekely, Lea I.	Microbiologist
Varrato, Wilma	Computer Programmer
Hancock, Kathryn	Statistical Assistant

Office of Biohazard Safety

Hellman, Alfred	Scientist Director, Chief
Twardzik, Dan	Chemist (Biochemistry)
West, David L.	San. Engineer

Biohazards Research Section

Fowler, Arnold	Sr. Scientist, Head
Allen, Patton T.	Microbiologist
Strickland, James E.	Chemist
Reed, Carl	Health Services Officer
Hegamyer, G.	Sr. Asst. Health Service Officer
Errico, Philip	Chemist
Harris, Cordia	Bio. Lab. Technician (Microbiologist)

Collaborative Research Branch

Office of the Chief

Manaker, Robert A.	Research Microbiologist, Acting Chief
Duff, James T.	Research Microbiologist

RNA Virus Studies Section

Woods, Wilna	Research Microbiologist, Head

DNA Virus Studies Section

Guss, Maurice	Microbiologist, Acting Head

Clinical Studies Section

Lavrin, David	Expert, Acting Head

Breast Cancer Virus Studies Section

Gaylord, Clarice	Geneticist, Acting Head

Cocarcinogenesis Studies Section

McQueen, James	Vet. Officer, Director, Acting Head

Laboratory of DNA Tumor Viruses

Office of the Chief

Manaker, Robert A.	Research Microbiologist, Chief
Hampar, Berge	Dent. Director, Asst. Chief

Microbiology Section

Hampar, Berge	Dent. Director, Head
Brown, Ashley M.	Sr. Dent. Surgeon
Showalter, Stephen	Biologist

Virus Tumor Biochemistry Section

Vande Woude, George	Research Chemist, Head
Ascione, Richard	Research Chemist

Lab. of DNA Tumor Viruses Branch (continued)

Virus Tumor Biochemistry Section (continued

Ebert, Paul S.	Research Chemist
Graham, Bettie J.	Staff Fellow
Oskarsson, Marianne	Chemist
Robey, William G.	Research Chemist
Stansly, Pauline	Biologist
Madden, Mary Jane	Microbiologist
Ortego, Harry	Microbiologist

Primate Virus Section (FCRC)

Ablashi, Dharam V.	Microbiologist, Head
Easton, John M.	Medical Director
Armstrong, Gary	Biologist

Cell Physiology Section

Hatanaka, Massakazu	Expert, Head

Virus Tumor Biology Section

Khoury, George	Surgeon, Head
Lai, Ching Juh	Expert
Segal, Shoshana	Microbiology
Aloni, Yosef	Visiting Scientist
Dhar, Ravi	Visiting Scientist
Holmgren, Kunig	Biological Lab. Tech. (Microbiologist)

Laboratory of Tumor Virus Genetics

Office of the Chief

Scolnick, Edward	Medical Officer (Res.), Head
Parks, Wade	Sr. Surgeon, Assoc. Chief
Chopra, Harish	Research Microbiologist
Sawdon, Margaret	Biological Lab. Technician

Viral Biochemistry Section

Bassin, Robert	Microbiologist, Head
Gerwin, Brenda I.	Chemist
Peebles, Paul T.	Sr. Surgeon

Lab. of Tumor Virus Genetics (continued)

Viral Biochemistry Section (continued)

Rein, Alan	Expert
Papageorge, Alex	Biologist
Smith, Susan	Microbiologist

Viral Immunogenetics Section

Parks, Wade	Sr. Surgeon, Head
Marciani, D.	Expert
Bader, John	Research Microbiologist
Papas, Takas	Research Chemist
Young, Howard	Staff Fellow
Brown, Nancy	Research Biologist
Pry, Thomas	Microbiologist
Ray, David	Research Chemist
Wolford, Ronald	Microbiologist
Chen, Claudia	Biologist
Hubbell, Elizabeth	Microbiologist
Noon, Marion	Biologist

Laboratory of RNA Tumor Viruses

Office of the Chief

Huebner, Robert	Medical Director, Chief
Arnstein, Paul	Vet. Office Director
Huebner, Harriet	Program Analyst
Hill, Paul	Supv. Bio. Lab. Tech. (Microbiologist)
Trimmer, Roy	Bio. Lab. Tech. (Microbiologist)
Wolford, Nancy	Biologist
Bare, Alton	Bio. Lab. Tech. (Animal)
Galblum, Sophie	Statistical Assistant
Hill, Richard	Biological Lab. Tech. (Genetics)
Kim, Gun-Hyuk	Biologist
Knieley, Charles	Biological Lab. Tech. (Microbiologist)
Scheibi, Allan	Biological Lab. Tech. (Microbiologist)
Smallwood, Herbert	Biological Lab. Tech. (Microbiologist)
Toliver, James	Biological Lab. Tech. (Microbiologist)

Molecular Biology Section

Aaronson, Stuart	Sr. Surgeon, Head
Reynolds, Roberta K.	Microbiologist
Porzig, Klaus	Surgeon
Hino, Shigeo	Expert

Lab. of RNA Tumor Viruses (continued)

Molecular Biology Section (continued)

Schrecker, Anthony	Research Chemist (Organic)
Dunn, Claire	Biologist
Long, Linda	Biologist

Viral Genetics Section

Stephenson, John	Microbiologist, Acting Head
Tronick, Steven R.	Microbiologist
Golub, Marjorie	Biologist

Animal Virology & Field Studies Section

Sarma, Padman	Research Microbiologist, Head

Viral Immunotherapy Section

Pearson, John	Microbiologist
Klein, David	Expert
Dahlberg, John	Microbiologist

Virus & Disease Modification Section

Chirigos, Michael	Research Chemist, Head
Merwin, Ruth M.	Research Microbiologist
Papadopoulos, Nicholas	Expert
Stylos, William	Expert
Meade, Bruce	Biologist
Schultz, Richard	Microbiologist

Viral Immunology Section

Kelloff, Gary	Sr. Surgeon, Head
Kende, Meir	Expert

Laboratory of Viral Carcinogenesis

Office of the Chief

Todaro, George J.	Medical Officer, Chief
Fischinger, Peter	Sr. Surgeon, Asst. Chief
Biggar, Robert J.	Sr. Surgeon

Laboratory of Viral Carcinogenesis (continued)

Office of the Chief (continued)

Leiseca, Sergio A.	Research Biologist
Levine, Paul H.	Sr. Surgeon
Plata, Ernest J.	Microbiologist

Virus Control Section

Fischinger, Peter	Senior Surgeon, Head
Haapala, Daniel K.	Microbiologist
Nomura, Shigeko	Visiting Scientist
Phillips, Leo	Microbiologist
Blevins, Charlotte	Microbiologist
Dunlop, Nancy	Microbiologist
Dunn, Karen	Bio. Lab. Tech. (Microbiologist)
Park, James	Chemist
Thornton, Arthur	Bio. Lab. Tech. (Microbiologist)

Immunology Section

Aoki, Tadao	Medical Officer, Head
Brandchaft, Phyllis	Microbioloigst
Kvedar, John P.	Bio. Technologist
Bushar, Grace	Biologist
Shaffer, Thomas	Bio. Lab. Tech. (Microbiologist)
Wallins, Mary Jane	Biologist

Ultrastructural Studies Section

Heine, Ursula	Research Microbiologist, Head
Suskind, Gerald	Medical Director
Elliott, Benjamin F., Jr.	Bio. Lab. Tech. (Microbiologist)
Luetzeler, Josef	Visiting Scientist
Collins, Fulmer	Bio. Lab. Tech. (Microbiologist)
Mangulies, Inger	Bio. Lab. Tech. (Microbiologist)
Schafer, Millie	Chemist
Wendel, Edmund	Biologist

Cell Biology Section

Boone, Charles W.	Medical Officer, Head
O'Brien, Stephen J.	Biologist (General)
Paranjpe, Meera	Biologist (General)
Takeichi, Noritoshi	Expert
Koegel, Robert J.	Research Chemist

Lab. of Viral Carcinogenesis Branch (continued)

Cell Biology Section (continued)

Eaton, Sol Del Ande	Chemist
Austin, Faye	Chemist
Simonson, Janice	Microbiologist

Viral Leukemia & Lymphoma Section

Todaro, George	Medical Officer, Head
Benveniste, Raoul E.	Microbiologist
Bonner, Tom I.	Expert
Callahan, Robert	Microbiologist
Marshall, Thomas	Expert
Sherr, Charles J.	Surgeon
Fedele, Louis	Biologist
Harvey, Estelle	Biologist
Meyer, Charlotte	Biologist
Nalewaik, Robert	Microbiologist
Wilson, Gaye	Biologist

Tumor Virus Detection Section

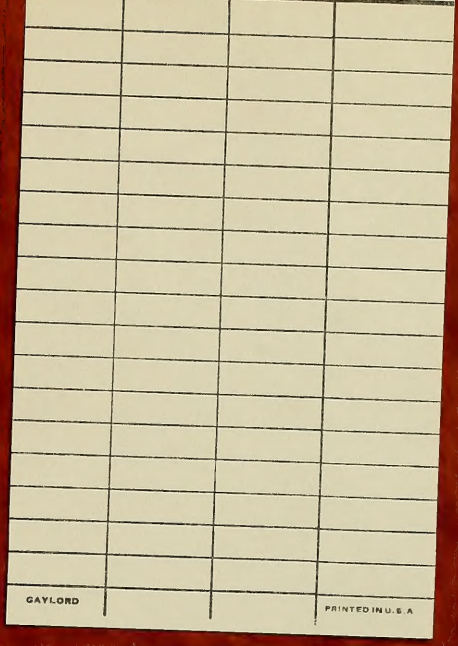
Schlom, Jeffrey	Microbiologist, Head

Viral Pathology Section

Donner, Ludvik	Visiting Scientist

NIH LIBRARY
4 0121 9793

* U.S. GOVERNMENT PRINTING OFFICE : 1977 O-720-079/7358



LIBRARY
NIH
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
3 1496 00185 6528